Winogradskyella ulvae sp. nov., an epiphyte of a Pacific seaweed, and emended descriptions of the genus *Winogradskyella* and *Winogradskyella thalassocola*, *Winogradskyella echinorum*, *Winogradskyella exilis* and *Winogradskyella eximia*

Olga I. Nedashkovskaya,¹ Andrey D. Kukhlevskiy^{2,3}
and Natalia V. Zhukova^{2,3}

Correspondence

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

²A.V. Zhirmunsky Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Science, Pal'chevskogo St. 17, 690032 Vladivostok, Russia

³Far Eastern Federal University, Sukhanova St. 8, 690950 Vladivostok, Russia

A Gram-negative, facultatively anaerobic, slightly halophilic bacterium that was motile by gliding, designated KMM 6390^T, was isolated from the coastal green alga *Ulva fenestrata* collected from the Sea of Japan. A comparative analysis of 16S rRNA gene sequences revealed that strain KMM 6390^T was a member of the genus *Winogradskyella*, with *Winogradskyella echinorum* KMM 6211^T as its closest relative (97.8% 16S rRNA gene sequence similarity). 16S rRNA gene sequence similarities between the isolate and other members of the genus *Winogradskyella* were 95.4–96.7%. DNA–DNA relatedness between the isolate and *W. echinorum* KMM 6211^T was 27%. Strain KMM 6390^T was able to grow with 0.5–4.0% NaCl and at 4–37 °C. The isolate decomposed casein, gelatin, starch and DNA and produced acid from galactose, D-glucose, lactose and maltose. The major fatty acids were iso-C_{15:0}, iso-C_{15:1}, anteiso-C_{15:0} and C_{15:0}. The polar lipid profile comprised phosphatidylethanolamine and two unknown aminolipids. The DNA G + C content was 34.2 mol%. On the basis of phylogenetic, chemotaxonomic and phenotypic investigations, strain KMM 6390^T represents a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella ulvae* sp. nov. is proposed; the type strain is KMM 6390^T (=KCTC 23626^T=LMG 26444^T). Emended descriptions of the genus *Winogradskyella* and of *W. thalassocola*, *W. echinorum*, *W. exilis* and *W. eximia* are also provided.

The genus *Winogradskyella*, a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002; Bernardet, 2011), was proposed by Nedashkovskaya *et al.* (2005) on the basis of phylogenetic, phenotypic and chemotaxonomic analysis of three isolates. *Winogradskyella thalassocola* is the type species of the genus. At the time of writing, there were nine species of the genus *Winogradskyella* with validly published names (Ivanova *et al.*, 2010; Lau *et al.*, 2005; Kim & Nedashkovskaya, 2010; Nedashkovskaya *et al.*, 2005, 2009; Romanenko *et al.*, 2009; Pinhassi *et al.*, 2009). An emended description of the genus *Winogradskyella* was proposed by Ivanova *et al.* (2010). Representatives of this genus have been

isolated from a variety of marine environments. Some are associated with the green algae *Acrosiphonia sonderi* and *Ulva linza* and with the brown algae *Chorda filum* and *Laminaria saccharina* (Marshall *et al.*, 2006; Nedashkovskaya *et al.*, 2005; Staufenberger *et al.*, 2008).

Strain KMM 6390^T was isolated from the green alga *Ulva fenestrata* collected from Troitsa Bay, Gulf of Peter the Great, Sea of Japan. For isolation, 0.1 ml seaweed homogenate was transferred to marine agar 2216 (MA; Difco). After primary isolation and purification, the strain was cultivated at 28 °C on the same medium and stored at –80 °C in marine broth (Difco) supplemented with 20% (v/v) glycerol.

DNA was extracted from 0.1–0.2 g wet cells using the extraction protocol of Sambrook & Russell (2001). PCR

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 6390^T is HQ456127.

was carried out using the universal oligonucleotide primers 11F and 1492R, as described by Lane (1991), and the GeneAmp PCR System 9700 (Applied Biosystems). PCR amplicons were used as templates for sequencing amplification using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems). The purified sequencing products were analysed by electrophoresis on a 50-cm capillary array of an ABI Prism 3130 DNA sequencer and the sequence was assembled with SeqScape version 2.6 (Applied Biosystems). After aligning the sequence with related sequences obtained from public databases, a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) using MEGA version 4 (Tamura *et al.*, 2007). Phylogenetic distances were calculated using the two-parameter model (Kimura, 1980). Maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) analyses were also carried out using the PHYLIP package (Felsenstein, 1993). Bootstrapping analysis with 1000 resamplings was undertaken to test the statistical reliability of the topology of the neighbour-joining tree (Felsenstein, 1985). An almost-complete 16S rRNA gene sequence (1406 bp) of strain KMM 6390^T was determined. Phylogenetic analysis revealed that strain KMM 6390^T was a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes* and formed a distinct evolutionary lineage within the genus *Winogradskyella* (Fig. 1). The neighbour-joining, maximum-likelihood and maximum-parsimony trees showed identical topologies (data not shown). The nearest neighbour of the isolate was *Winogradskyella echinorum* KMM 6211^T (97.8 % 16S rRNA gene sequence similarity). 16S rRNA gene sequence similarities between strain KMM 6390^T and the type strains of other recognized species of the genus *Winogradskyella* were 95.4–96.7 %.

DNA was isolated following the method of Marmur (1961) and the DNA G + C content was determined by the thermal denaturation method of Marmur & Doty (1962). The DNA

G + C content of strain KMM 6390^T was 34.2 mol%, which is in the range reported for members of the genus *Winogradskyella* (30.1–36.3 mol%; Ivanova *et al.*, 2010; Lau *et al.*, 2005; Kim & Nedashkovskaya, 2010; Nedashkovskaya *et al.*, 2005, 2009; Pinhassi *et al.*, 2009). DNA–DNA hybridization was performed spectrophotometrically using the initial renaturation rate method of De Ley *et al.* (1970). DNA–DNA relatedness between strain KMM 6390^T and *W. echinorum* KMM 6211^T was 27 %, which indicated that the isolate represented a distinct and novel species of the genus *Winogradskyella* (Wayne *et al.*, 1987).

For whole-cell fatty acid and polar lipid analysis, strain KMM 6390^T, *W. thalassocola* KMM 3907^T, *W. echinorum* KMM 6211^T, *Winogradskyella exilis* 022-2-26^T and *Winogradskyella eximia* KMM 6244^T were grown at 28 °C for 48 h on MA. Cellular fatty acid methyl esters (FAMES) were prepared according to the methods described by Sasser (1990) using the standard protocol of the Sherlock Microbial Identification System (version 6.0, MIDI) and analysed using a GC-21A chromatograph (Shimadzu) equipped with a fused-silica capillary column (30 m × 0.25 mm) coated with Supelcowax-10 and SPB-5 phases (Supelco) at 210 °C. FAMES were identified by using equivalent chain-length measurements and comparing the retention times to those of authentic standards. FAMES were also analysed by GC-MS (QP5050A; Shimadzu) equipped with an MDN-5S capillary column (30 m ± 0.25 mm), the temperature programme was from 140 to 250 °C, at a rate of 2 °C min⁻¹. The prevalent fatty acids of strain KMM 6390^T were iso-C_{15:0} (33.4 %), iso-C_{15:1} (16.1 %), anteiso-C_{15:0} (12.8 %) and C_{15:0} (10.3 %) (Table 1). The fatty acids of strain KMM 6390^T were consistent with those reported for the genus *Winogradskyella* (Kim & Nedashkovskaya, 2010; Nedashkovskaya *et al.*, 2005; Lau *et al.*, 2005) and the reference strains included in this study (Table 1). However, there were significant differences in the proportions of some fatty acids between strain KMM

Fig. 1. Neighbour-joining phylogenetic tree showing the relationships of strain KMM 6390^T, members of the genus *Winogradskyella* and other representatives of the family *Flavobacteriaceae*. Bootstrap values (>70 %) based on 1000 resampled datasets are shown at branch nodes. *Zobellia amurskyensis* KMM 3526^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 1. Fatty acid compositions of strain KMM 6390^T and its closest phylogenetic neighbours in the genus *Winogradskyella*

Strains: 1, *Winogradskyella ulvae* sp. nov. KMM 6390^T; 2, *W. echinorum* KMM 6211^T; 3, *W. thalassocola* KMM 3907^T; 4, *W. exilis* 022-2-26^T; 5, *W. eximia* KMM 3944^T. All data were taken from this study. Fatty acids amounting to <1 % in all strains are not shown. tr, Trace (<1 %); –, not detected.

Fatty acid (%)	1	2	3	4	5
iso-C _{13:0}	—	tr	1.2	—	—
iso-C _{14:0}	1.6	tr	tr	tr	1.2
C _{14:0}	tr	tr	tr	tr	1.3
C _{15:0}	10.3	8.3	10.2	5.0	11.9
C _{15:1} ω6c	tr	tr	4.5	tr	3.6
C _{15:1} ω8c	tr	1.5	1.4	—	1.5
iso-C _{15:0}	33.4	21.1	10.9	38.9	28.5
anteiso-C _{15:0}	12.8	1.9	9.6	1.3	9.1
iso-C _{15:1}	16.1	32.6	16.4	28.3	14.8
iso-C _{15:0} 3-OH	2.5	8.0	8.0	3.1	1.3
iso-C _{15:0} 2-OH	3.2	9.6	4.0	8.0	1.5
C _{15:0} 3-OH	—	tr	1.4	—	—
C _{16:0}	2.3	1.2	1.0	1.0	2.5
iso-C _{16:0}	3.0	tr	1.0	2.2	2.8
anteiso-C _{16:0}	tr	—	—	—	1.6
iso-C _{16:1}	2.0	tr	—	—	2.7
C _{16:1} ω7c	1.5	tr	5.2	tr	6.8
C _{16:1} ω5c	—	—	1.0	—	tr
iso-C _{16:0} 3-OH	3.4	1.7	13.2	2.7	2.6
C _{16:0} 3-OH	—	—	1.0	—	—
C _{17:1} ω6c	—	—	2.6	—	tr
iso-C _{17:1}	1.2	1.6	—	—	1.8
iso-C _{17:0} 3-OH	1.9	3.3	3.4	6.8	1.0

6390^T and its closest phylogenetic neighbour, *W. echinorum* KMM 6211^T (i.e. anteiso-C_{15:0} and iso-C_{15:1}) and the type strain of the type species of the genus, *W. thalassocola* KMM 3907^T (i.e. C_{15:1}ω6c, iso-C_{15:0} and iso-C_{16:0} 3-OH) (Table 1). Differences in fatty acid composition were also observed between the isolate and *W. exilis* 022-2-26^T and *W. eximia* KMM 3944^T (Table 1). The distinctiveness of the fatty acid composition of strain KMM 6390^T confirmed

that it could be considered as a representative of a novel species of the genus *Winogradskyella*.

The polar lipids of strain KMM 6390^T and the reference strains were extracted using the chloroform/methanol extraction method of Bligh & Dyer (1959). Two-dimensional TLC of polar lipids was carried out on silica gel 60 F254 (10 × 10 cm; Merck) using chloroform/methanol/water (65:25:4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, by vol.) in the second dimension (Collins & Shah, 1984). The spray reagent used to reveal the spots was molybdophosphoric acid. The polar lipid profile of strain KMM 6390^T was composed of phosphatidylethanolamine and two unknown aminolipids, which was in line with the reference strains except that *W. eximia* KMM 3944^T contained only one unknown amino-lipid (Fig. 2).

Isoprenoid quinone composition of strain KMM 6390^T was characterized by HPLC (LC-10A; Shimadzu) using a reversed-phase Supelcosil LC-18 column (15 cm × 4.6 mm) and acetonitrile/2-propanol (65:35, v/v) as a mobile phase at a flow rate of 0.5 ml min⁻¹. The column was kept at 40 °C. Menaquinones were detected by monitoring at 270 nm and were identified by comparing them with known quinones from *Bizionia paragorgiae* KMM 6029^T. The sole respiratory quinone of the isolate was menaquinone MK-6, which is in line with all other members of the family *Flavobacteriaceae*.

The physiological, morphological and biochemical properties of strain KMM 6390^T, *W. thalassocola* KMM 3907^T, *W. echinorum* KMM 6211^T, *W. exilis* 022-2-26^T and *W. eximia* KMM 3944^T were tested as described previously (Nedashkovskaya *et al.*, 2003, 2004). Oxidative and fermentative utilization of glucose were determined using the medium of Hugh and Leifson modified for marine bacteria (Lemos *et al.*, 1985). The presence of oxidase was tested using *N,N,N',N'*-tetramethyl-*p*-phenylenediamine and hydrogen sulfide production was determined using sodium thiosulphate according to Smibert & Krieg (1994). The isolate and the reference strains were also examined using the API 20 E, API 20 NE and API ZYM systems

Fig. 2. Two-dimensional TLC of the total polar lipids of strain KMM 6390^T (a), *W. thalassocola* KMM 3907^T (b), *W. echinorum* KMM 6211^T (c), *W. eximia* KMM 3944^T (d) and *W. exilis* 022-2-26^T (e). First dimension, chloroform/methanol/water (65:25:4, by vol.); second dimension, chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). The spray reagent was molybdophosphoric acid. AL, Unknown aminolipid; PE, phosphatidylethanolamine.

(bioMérieux), according to the manufacturer's instructions except that the galleries were incubated at 28 °C.

The physiological and biochemical characteristics of strain KMM 6390^T are given in the species description and Table 2. The isolate shared many phenotypic properties with members of the genus *Winogradskyella*. It displayed gliding motility, produced alkaline and acid phosphatases, catalase, gelatinase and oxidase and required NaCl or seawater for growth. However, strain KMM 6390^T clearly differed from the recognized species of the genus *Winogradskyella* by its ability to form acid from D-galactose and lactose under aerobic conditions, to utilize lactose and to ferment D-glucose (only *W. exilis* 022-2-26^T shared this characteristic). In addition, features such as the type of metabolism, production of hydrogen sulphide, absence of α -chymotrypsin and presence of *N*-acetyl- β -glucosaminidase, hydrolysis of agar, starch and Tweens 40 and 80, and production of acid from D-glucose and maltose may be helpful for separation of the novel strain from its closest relative, *W. echinorum* KMM 6211^T (Table 2).

On the basis of the results of the phylogenetic, genotypic, chemotaxonomic and phenotypic analyses, we suggest that strain KMM 6390^T be classified as a representative of a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella ulvae* sp. nov. is proposed.

Originally, members of the genus *Winogradskyella* were described as oxidase-positive and nitrate reductase-negative organisms that required NaCl or seawater for growth and could produce hydrogen sulphide. The genus description was emended subsequent to the description of *W. exilis* 022-2-26^T (Ivanova *et al.*, 2010) as this strain was reported to be oxidase-negative, nitrate reductase-positive, able to grow without NaCl or seawater and unable to produce hydrogen sulphide. We reinvestigated the phenotypic features of *W. exilis* 022-2-26^T in this study and found that it actually displayed oxidase activity and produced hydrogen sulphide. In addition, the polar lipid profiles of *W. exilis* 022-2-26^T, *W. thalassocola* KMM 3907^T, *W. echinorum* KMM 6211^T and *W. eximia* KMM 3944^T were determined in the present study. Hence, the emended descriptions of the genus *Winogradskyella* and these four species are given here.

Emended description of the genus *Winogradskyella* Nedashkovskaya *et al.* 2005 emend. Ivanova *et al.* 2010

The description is as given by Nedashkovskaya *et al.* (2005) and Ivanova *et al.* (2010) with the following amendments. Oxidase- and catalase-positive. Strictly aerobic or facultatively anaerobic. The polar lipid profile comprises phosphatidylethanolamine and one or two unknown aminolipids. The major respiratory quinone is menaquinone MK-6. The type species is *Winogradskyella thalassocola*.

Description of *Winogradskyella ulvae* sp. nov.

Winogradskyella ulvae (ul'vae. N.L. gen. n. *ulvae* of *Ulva*, the name of a genus of green algae, isolated from a frond surface of *Ulva*).

Cells are heterotrophic, facultatively anaerobic, Gram-negative rods (approximately 0.4–0.5 μ m wide and 1.2–2.7 μ m long) and motile by gliding. On marine agar, colonies are 2–3 mm in diameter, circular with entire edges, shiny, mucous, bright yellow and slightly sunken into the agar. Growth occurs at 4–37 °C (optimum 25–28 °C), at pH 5.5–10.0 (optimum pH 7.5) and with 0.5–4.0 % NaCl (optimum 0.5 %). Catalase and oxidase activities are present. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are absent. D-Glucose is fermented. Aesculin, gelatin, casein, starch and DNA are hydrolysed, agar and Tweens 40 and 80 are weakly hydrolysed, but Tween 20, urea, cellulose (CM-cellulose and filter paper) and chitin are not hydrolysed. Acid is produced from D-galactose, D-glucose, lactose, maltose and amygdalin, but not from L-arabinose, cellobiose, D-fructose, mannose, melibiose, raffinose, L-rhamnose, sucrose, xylose, *N*-acetylglucosamine, glycerol or mannitol. Mannose and sucrose are utilized, but L-arabinose, *N*-acetylglucosamine, inositol, mannitol, sorbitol, adipate, caprate, citrate, gluconate, malate, malonate and phenylacetate are not utilized. With API ZYM, positive for esterase lipase (C8), leucine arylamidase, valine arylamidase, alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β -galactosidase, weakly positive for esterase (C4), cystine arylamidase and *N*-acetyl- β -glucosaminidase, but negative for lipase (C14), trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α - and β -glucosidase, α -mannosidase and α -fucosidase. Flexirubin-type pigments are not formed. Nitrate is not reduced. Hydrogen sulphide is produced weakly, but indole and acetoin are not. Susceptible to (μ g, unless otherwise indicated) ampicillin (10), benzylpenicillin (10 U), carbenicillin (100), cefalexin (30), cefazolin (30), chloramphenicol (30), erythromycin (15), doxycycline (10), lincomycin (15), nalidixic acid (30), ofloxacin (5), oleandomycin (15), rifampicin (5), tetracycline (5) and vancomycin (30) but resistant to gentamicin (10), kanamycin (30), neomycin (30), oxacillin (10), polymyxin B (300 U) and streptomycin (30). The major fatty acids (>10 %) are iso-C_{15:0}, iso-C_{15:1}, anteiso-C_{15:0} and C_{15:0}. The polar lipid profile comprises phosphatidylethanolamine and two unknown aminolipids. The sole respiratory quinone is MK-6.

The type strain, KMM 6390^T (=KCTC 23626^T=LMG 26444^T), was isolated from the green alga *U. fenestrata*, collected from Troitsa Bay, Sea of Japan. The DNA G+C content of the type strain is 34.2 mol%.

Emended description of *Winogradskyella thalassocola* Nedashkovskaya *et al.* 2005

In addition to the characteristics listed in the emended description of the genus above, the description is as given by Nedashkovskaya *et al.* (2005) with the following amendments. Cells are susceptible to cefalexin, cefazolin, chloramphenicol, doxycycline, erythromycin, nalidixic acid, ofloxacin, rifampicin and vancomycin and resistant to

Table 2. Phenotypic characteristics differentiating strain KMM 6390^T from members of the genus *Winogradskyella*

Strains: 1, *Winogradskyella ulvae* sp. nov. KMM 6390^T (this study); 2, *W. echinorum* KMM 6211^T (Nedashkovskaya *et al.*, 2009); 3, *W. thalassocola* KMM 3907^T (Nedashkovskaya *et al.*, 2005; this study); 4, *W. arenosi* KMM 3968^T (Romanenko *et al.*, 2009); 5, *W. epiphytica* KMM 3906^T (Nedashkovskaya *et al.*, 2005; this study); 6, *W. exilis* 022-2-26^T (Ivanova *et al.*, 2010; this study); 7, *W. eximia* KMM 3944^T (Nedashkovskaya *et al.*, 2005; this study); 8, *W. pacifica* KMM 6019^T (Kim & Nedashkovskaya, 2010); 9, *W. poriferorum* UST030701-295^T (Lau *et al.*, 2005); 10, *W. rapida* SCB36^T (Pinhassi *et al.*, 2009). All strains are positive for gliding motility, oxidase, catalase, alkaline phosphatase, esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase, requirement for Na⁺ ions for growth and gelatin hydrolysis. All strains are negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, β -glucuronidase, α -mannosidase and α -fucosidase, production of flexirubin-type pigments, indole and acetoin, hydrolysis of chitin, acid production from L-arabinose, melibiose, L-rhamnose, D-xylose, adonitol, dulcitol, inositol, sorbitol and citrate and utilization of L-arabinose, malonate and citrate. +, Positive; (+), weakly positive, –, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10
Type of metabolism*	F	A	A	A	A	F	A	A	A	A
Fermentation of D-glucose	+	–	–	–	–	+	–	–	–	–
Nitrate reductase	–	–	–	–	–	+	–	–	–	–
Hydrogen sulphide production	(+)	–	–	+	–	+	–	+	–	+
Acetoin and indole production	–	–	–	–	–	–	–	–	+	–
Degradation of:										
Aesculin	+	+	+	+	–	–	+	+	+	–
Agar	(+)	–	+	–	+	–	+	–	–	–
Casein	+	+	–	–	–	–	+	+	–	+
Starch	+	–	–	+	–	+	+	+	–	+
Tween 20	–	–	–	+	+	–	+	–	+	+
Tween 40	(+)	–	+	+	+	+	+	–	+	+
Tween 80	(+)	–	–	+	+	+	–	–	+	+
DNA	+	+	–	–	+	–	–	–	+	–
Urea	–	–	–	–	–	+	–	–	–	–
Growth at/with:										
37 °C	+	+	–	–	+	+	–	–	+	–
44 °C	–	–	–	–	–	–	–	–	+	–
8 % NaCl	–	–	+	+	+	–	–	–	–	+
Acid production from:										
Cellobiose	–	–	+	–	–	–	–	+	–	–
D-Galactose, lactose	+	–	–	–	–	–	–	–	–	–
D-Glucose, maltose	+	–	+	+	–	–	+	+	–	+
Sucrose	–	–	–	–	–	–	+	–	–	–
Mannitol	–	–	–	+	–	–	+	–	–	+
Utilization of:										
D-Glucose	+	+	+	+	–	–	+	+	–	+
Lactose	+	–	–	–	–	–	–	–	–	–
D-Mannose	+	+	+	+	–	–	+	–	–	+
Sucrose	+	–	–	–	–	–	+	–	–	–
Mannitol	–	–	–	+	–	–	+	+	–	+
Inositol, sorbitol	–	–	–	–	–	–	–	+	–	–
Enzyme activities (API ZYM)										
Esterase (C4)	(+)	+	–	+	+	+	+	+	+	+
Leucine arylamidase	+	+	+	+	+	+	–	+	+	+
Cystine arylamidase	(+)	+	–	+	–	–	–	+	+	–
Valine arylamidase	+	+	–	+	+	+	+	+	+	+
Lipase (C14), trypsin	–	–	–	–	–	–	–	–	+	+
α -Chymotrypsin	–	+	–	–	–	–	–	–	+	–
α -Galactosidase	–	–	–	–	–	–	+	–	–	–
β -Galactosidase	+	+	+	–	–	–	–	–	–	–
α -Glucosidase	–	–	+	+	–	–	–	–	–	+
β -Glucosidase	–	–	+	–	–	–	–	–	–	–
N-Acetyl- β -glucosaminidase	(+)	–	+	–	–	–	–	–	–	–

*A, Strictly aerobic; F, facultatively anaerobic.

oxacillin. Aesculin is hydrolysed. With API ZYM, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α - and β -glucosidase and *N*-acetyl- β -glucosaminidase, but negative for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. The polar lipid profile of the type strain comprises phosphatidylethanolamine and two unknown aminolipids.

The type strain is DSM 15363^T (=KCTC 12221^T=KMM 3907^T=LMG 22492^T).

Emended description of *Winogradskyella echinorum* Nedashkovskaya et al. 2009

In addition to the characteristics listed in the emended description of the genus above, the description is as given by Nedashkovskaya et al. (2009) with the following amendments. The polar lipid profile of the type strain comprises phosphatidylethanolamine and two unknown aminolipids.

The type strain is KCTC 22026^T (=KMM 6211^T=LMG 24757^T).

Emended description of *Winogradskyella exilis* Ivanova et al. 2010

In addition to the characteristics listed in the emended description of the genus above, the description is as given by Ivanova et al. (2010) with the following amendments. Cells are facultatively anaerobic. Acid is not produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, melibiose, raffinose, L-rhamnose, ribose, sorbose, sucrose, xylose, glycerol, mannitol, acetate or *N*-acetylglucosamine. Hydrogen sulphide is produced. The polar lipid profile of the type strain comprises phosphatidylethanolamine and two unknown aminolipids.

The type strain is 022-2-26^T (=CIP 109976^T=KMM 6013^T).

Emended description of *Winogradskyella eximia* Nedashkovskaya et al. 2005

In addition to the characteristics listed in the emended description of the genus above, the description is as given by Nedashkovskaya et al. (2005) with the following amendments. Aesculin is hydrolysed. With API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, acid phosphatase, α -galactosidase and naphthol-AS-BI-phosphohydrolase activities, but negative for lipase (C14), leucine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The polar lipid profile of the type strain comprises phosphatidylethanolamine and one unknown aminolipid.

The type strain is KCTC 12219^T (=KMM 3944^T=LMG 22474^T).

Acknowledgements

This research was supported by grants from the Russian Foundation for Basic Research (RFBR) (grant no. 09-04-00420), the Presidium of the Russian Academy of Sciences 'Molecular and Cell Biology' and the Government of Russian Federation for the state support of scientific investigations conducted under the guidance of leading researchers at Russian higher education institutions (agreement no. 11.G34.31.0010).

References

- Bernardet, J.-F. (2011). Family I. *Flavobacteriaceae* Reichenbach 1992. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 4, pp. 106–111. Edited by N. J. Krieg, W. Ludwig, W. B. Whitman, B. P. Hedlund, B. J. Paster, J. T. Staley, N. Ward, D. Brown & A. Parte. New York: Springer.
- Bernardet, J.-F., Nakagawa, Y., Holmes, B. & Subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes (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.
- Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37, 911–917.
- Collins, M. D. & Shah, H. M. (1984). Fatty acid, menaquinone and polar lipid composition of *Rothia dentosaccharosa*. *Arch Microbiol* 137, 247–249.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* 12, 133–142.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Ivanova, E. P., Christen, R., Gorshkova, N. M., Zhukova, N. V., Kurilenko, V. V., Crawford, R. J. & Mikhailov, V. V. (2010). *Winogradskyella exilis* sp. nov., isolated from the starfish *Stellaster equestris*, and emended description of the genus *Winogradskyella*. *Int J Syst Evol Microbiol* 60, 1577–1580.
- Kim, S. B. & Nedashkovskaya, O. I. (2010). *Winogradskyella pacifica* sp. nov., a marine bacterium of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* 60, 1948–1951.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16, 111–120.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* 18, 1–32.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lau, S. C. K., Tsoi, M. M. Y., Li, X., Plakhotnikova, I., Dobretsov, S., Lau, K. W. K., Wu, M., Wong, P.-K., Pawlik, J. R. & Qian, P.-Y. (2005). *Winogradskyella poriferorum* sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a sponge in the Bahamas. *Int J Syst Evol Microbiol* 55, 1589–1592.

- Lemos, M. L., Toranzo, A. E. & Barja, J. L. (1985). Modified medium for the oxidation-fermentation test in the identification of marine bacteria. *Appl Environ Microbiol* **49**, 1541–1543.
- 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.
- Marshall, K., Joint, I., Callow, M. E. & Callow, J. A. (2006). Effect of marine bacterial isolates on the growth and morphology of axenic plantlets of the green alga *Ulva linza*. *Microb Ecol* **52**, 302–310.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, A. M., Rohde, M., Zhukova, N. V., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2003). *Mesonina algae* gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the green alga *Acrosiphonia sonderi* (Kütz) Kornm. *Int J Syst Evol Microbiol* **53**, 1967–1971.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Rhee, M. S., Lysenko, A. M., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2004). *Ulvibacter litoralis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **54**, 119–123.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Snauwaert, C., Vancanneyt, M., Swings, J., Kim, K. O., Lysenko, A. M., Rohde, M. & other authors (2005). *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **55**, 49–55.
- Nedashkovskaya, O. I., Vancanneyt, M., Kim, S. B. & Zhukova, N. V. (2009). *Winogradskyella echinorum* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from the sea urchin *Strongylocentrotus intermedius*. *Int J Syst Evol Microbiol* **59**, 1465–1468.
- Pinhassi, J., Nedashkovskaya, O. I., Hagström, Å. & Vancanneyt, M. (2009). *Winogradskyella rapida* sp. nov., isolated from protein-enriched seawater. *Int J Syst Evol Microbiol* **59**, 2180–2184.
- Romanenko, L. A., Tanaka, N., Frolova, G. M. & Mikhailov, V. V. (2009). *Winogradskyella arenosi* sp. nov., a member of the family *Flavobacteriaceae* isolated from marine sediments from the Sea of Japan. *Int J Syst Evol Microbiol* **59**, 1443–1446.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Staufenberger, T., Thiel, V., Wiese, J. & Imhoff, J. F. (2008). Phylogenetic analysis of bacteria associated with *Laminaria saccharina*. *FEMS Microbiol Ecol* **64**, 65–77.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, P., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.