Winogradskyella pacifica sp. nov., a marine bacterium of the family *Flavobacteriaceae*

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A novel strain, designated KMM 6019^T, was isolated from coastal seawater and subjected to taxonomic examination using a polyphasic approach. A comparative analysis of 16S rRNA gene sequences revealed that strain KMM 6019^T formed a distinct phyletic line within the genus *Winogradskyella*, a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. The 16S rRNA gene sequence similarity between the novel isolate and the type strains of the recognized species of the genus *Winogradskyella* was 94.0–97.8%. *Winogradskyella thalassocola* KMM 3907^T was the closest relative, with 97.8% sequence similarity. The predominant fatty acids of strain KMM 6019^T were iso-C_{15:0}, iso-C_{15:1}, anteiso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{16:0} 3-OH, C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, which are characteristic for members of the genus *Winogradskyella*. The DNA G + C content was 35.3 mol%. Strain KMM 6019^T moved by gliding and grew with 1–5% NaCl and at 4–30 °C. The novel strain degraded gelatin, casein and starch and produced acid from cellobiose, D-glucose and maltose. Strain KMM 6019^T could clearly be differentiated from the other *Winogradskyella* species by the ability to utilize inositol and sorbitol and to produce hydrogen sulphide. On the basis of phylogenetic and phenotypic findings, strain KMM 6019^T is considered to represent a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella pacifica* sp. nov. is proposed. The type strain is KMM 6019^T (=KCTC 22997^T=LMG 22568^T).

The genus *Winogradskyella* is a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002) and at the time of writing comprises seven recognized species: *W. arenosi*, *W. echinorum*, *W. epiphytica*, *W. eximia*, *W. poriferorum*, *W. rapida* and *W. thalassocola* (Lau *et al.*, 2005; Nedashkovskaya *et al.*, 2005, 2009; Romanenko *et al.*, 2009; Pinhassi *et al.*, 2009). To date, the genus *Winogradskyella* is represented by marine bacteria isolated from seawater, sediments, seaweed and animals. Phylogenetically, the genus *Winogradskyella* forms a cluster with the genera *Psychroserpens*, *Formosa*, *Bizionia*, *Lacinutrix* and *Gaetbulibacter*. In this work, we report the detailed taxonomic characterization of a novel marine bacterium belonging to the genus *Winogradskyella*.

Strain KMM 6019^T was isolated from a coastal seawater sample collected from Amursky Bay, Gulf of Peter the Great, the East Sea (also known as the Sea of Japan). For strain isolation, 0.1 ml seawater was transferred onto plates of 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.

The phylogenetic position of strain KMM 6019^T was determined from its almost-complete 16S rRNA gene sequence (1424 bp). Genomic DNA extraction, PCR and sequencing of 16S rRNA genes were performed by using previously described procedures (Cho *et al.*, 2006). The sequences obtained were aligned with those of representative members of selected genera belonging to the family *Flavobacteriaceae* by using PHYDIT version 3.1 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred by using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the two-parameter model (Kimura, 1980), and the trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) algorithms. Bootstrap analysis was performed with 1000 resampled datasets, using SEQBOOT and CONSENSE programs of the PHYLIP package.

A comparative analysis of the almost-complete 16S rRNA gene sequence of strain KMM 6019^T revealed that the strain formed a distinct evolutionary lineage within the genus *Winogradskyella*, a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes* (Fig. 1). The 16S

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Winogradskyella pacifica* KMM 6019^T is GQ181061.

Fig. 1. Neighbour-joining tree of the genus *Winogradskyella* and related taxa. The identical topology was obtained in maximum-likelihood and maximum-parsimony trees. Values at nodes are the bootstrap support (%) based on a 1000 resampled dataset. Bar, 0.01 substitutions per nucleotide position.

rRNA gene sequence similarity between the novel isolate and the type strains of the recognized species of the genus *Winogradskyella* was 94.0–97.8 %. The closest relative was *Winogradskyella thalassocola* KMM 3907^T, with 97.8 % sequence similarity. This value is lower than the recommended extreme value for species delineation (about 99 %) without determination of the degree of DNA–DNA reassociation between the relative bacterial strains (Stackebrandt & Ebers, 2006).

DNA was isolated following the method of Marmur (1961) and the DNA G + C content was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G + C content of KMM 6019^T was 35.3 mol%. This value is in the range of the DNA G + C content of the recognized *Winogradskyella* species (Table 1).

To determine whole-cell fatty acid composition by the MIDI procedure, strain KMM 6019^T was grown at 28 °C for 48 h on MA. The fatty acids accounting for more than 1 % of the total were iso-C_{15:0} (15.8 %), iso-C_{15:1} (11.7 %), anteiso-C_{15:0} (11.1 %), iso-C_{15:0} 3-OH (8.9 %), iso-C_{16:0} 3-OH (8.6 %), C_{15:0} (6.2 %), iso-C_{17:0} 3-OH (5.3 %), summed feature 3 comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (4.2 %), C_{15:1}ω6c (3.1 %), iso-C_{16:1} H (2.2 %), anteiso-C_{15:1} (2.1 %), iso-C_{14:0} (1.8 %), iso-C_{16:0} (1.4 %),

C_{15:0} 2-OH (1.4 %), iso-C_{17:1}ω9c (1.4 %), C_{17:1}ω6c (1.4 %), C_{15:0} 3-OH (1.3 %) and C_{17:0} 3-OH (1.3 %). The cellular fatty acids of strain KMM 6019^T are consistent with those reported for the recognized species of the genus *Winogradskyella* (Nedashkovskaya *et al.*, 2005, 2009; Lau *et al.*, 2005).

The physiological, morphological and biochemical characteristics of strain KMM 6019^T were studied as described previously (Nedashkovskaya *et al.*, 2003, 2004). API 20 E, API 20 NE and API ZYM galleries (bioMérieux) were also used for examination of the phenotypic features of the strain studied according to the manufacturer's instructions, except that the galleries were incubated at 28 °C.

The main physiological and biochemical characteristics of strain KMM 6019^T are given in the species description and in Table 1. The novel isolate shared many phenotypic properties in common with the *Winogradskyella* species. It was a strictly aerobic and motile-by-gliding organism that produced alkaline phosphatase, catalase, gelatinase and oxidase, and required NaCl or seawater for growth. However, it can clearly be differentiated from the recognized species of the genus *Winogradskyella* by its ability to utilize inositol and sorbitol and to produce hydrogen sulphide. Several traits such as hydrolysis of agar,

Table 1. Phenotypic characteristics differentiating strain KMM 6019^T from the recognized *Winogradskyella* species

Strains: 1, KMM 6019^T; 2, *W. thalassocola* KMM 3907^T; 3, *W. arenosi* KMM 3968^T; 4, *W. echinorum* KMM 6211^T; 5, *W. epiphytica* KMM 3906^T; 6, *W. eximia* KMM 3944^T; 7, *W. poriferorum* UST030701-295^T; 8, *W. rapida* SCB36^T. Data are from Lau *et al.* (2005), Nedashkovskaya *et al.* (2005, 2009), Pinhassi *et al.* (2009), Romanenko *et al.* (2009) and this study. All strains were positive for the following: respiratory metabolism; gliding motility; oxidase, catalase and alkaline phosphatase activities; a requirement for Na⁺ ions for growth; gelatin hydrolysis; susceptibility to carbenicillin and lincomycin; and resistance to benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. All strains were negative for the following: nitrate reductase activity; flexirubin-type pigments; indole and acetoin production; hydrolysis of urea and chitin; acid production from L-arabinose, D-galactose, D-lactose, melibiose, L-rhamnose, D-xylose, adonitol, dulcitol, inositol, sorbitol and citrate; and utilization of L-arabinose, D-lactose, malonate and citrate. ND, Data are not available.

Characteristic	1	2	3	4	5	6	7	8
Hydrogen sulphide production	+	–	+	–	–	–	–	+
Degradation of:								
Agar	–	+	–	–	+	+	–	–
Casein	+	–	–	+	–	+	–	+
Starch	+	–	+	–	–	+	–	+
Tween 20	–	–	+	–	+	+	+	+
Tween 40	–	+	+	–	+	+	+	+
Tween 80	–	–	+	–	+	–	+	+
DNA	–	–	–	+	+	–	+	–
Growth at/with:								
37 °C	–	–	–	+	+	–	+	–
44 °C	–	–	–	–	–	–	+	–
8 % NaCl	–	+	+	–	+	–	–	+
Acid production from:								
D-Glucose, maltose	+	+	+	–	–	+	–	+
Cellobiose	+	+	–	–	–	–	–	–
Sucrose	–	–	–	–	–	+	–	–
Mannitol	–	–	+	–	–	+	–	+
Utilization of:								
D-Glucose	+	+	+	+	–	+	–	+
D-Mannose	–	+	+	+	–	+	–	+
Sucrose	–	–	–	–	–	+	–	–
Mannitol	+	–	+	–	–	+	–	+
Inositol and sorbitol	+	–	–	–	–	–	–	–
Enzyme activities								
Esterase (C4), valine arylamidase and cystine arylamidase	+	–	+	+	ND	ND	+	+
Lipase (C14), trypsin	–	–	–	–	ND	ND	+	+
α -Chymotrypsin	–	–	–	+	ND	ND	+	–
β -Galactosidase	–	+	–	+	–	–	–	–
α -Glucosidase	–	+	+	–	ND	ND	–	+
β -Glucosidase, N-acetyl- β -glucosaminidase	–	+	–	–	ND	ND	–	–
Susceptibility to:								
Ampicillin	+	–	ND	–	+	–	+	+
Oleandomycin	+	+	ND	+	+	–	ND	+
Tetracycline	–	–	ND	–	+	+	+	+
DNA G + C content (mol%)	35.3	34.6	ND	33.6	35.2	36.1	32.8	36.3

casein, starch and Tween 40, growth with 8 % NaCl and utilization of D-mannose and mannitol could be useful for separation of the strain under study from its nearest phylogenetic neighbour, *W. thalassocola* KMM 3907^T (Table 1). The presence of esterase (C4), valine arylamidase and cystine arylamidase activities and the absence of β -galactosidase, α -glucosidase, β -glucosidase and N-acetyl- β -glucosaminidase activities, determined in KMM 6019^T cells using the API ZYM gallery, contrasted with the results for *W. thalassocola* KMM 3907^T (Table 1). It should be noted

that the latter strain has been described as β -galactosidase-negative (Nedashkovskaya *et al.*, 2005). This discrepancy could be due to the use of different methods to assay for β -galactosidase activity. Phenotypic characteristics differentiating strain KMM 6019^T from other *Winogradskyella* species are shown in Table 1.

Consequently, on the basis of phylogenetic, genotypic, chemotaxonomic and phenotypic data, strain KMM 6019^T should be classified as the type strain of a novel species

within the genus *Winogradskyella*, for which the name *Winogradskyella pacifica* sp. nov. is proposed.

Description of *Winogradskyella pacifica* sp. nov.

Winogradskyella pacifica (pa.ci'fi.ca. L. fem. adj. *pacifica* pacific, pertaining to the Pacific Ocean from where the type strain was isolated).

Cells are heterotrophic, strictly aerobic, motile by gliding, Gram-negative rods, 0.4–0.5 µm wide and 1.0–2.3 µm long. On marine agar, colonies are 2–3 mm in diameter, circular, shiny with entire edges and yellow-coloured. Growth occurs at 4–30 °C and with 1–5 % NaCl (optimum 1–3 % NaCl). Produces catalase and oxidase. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are absent. Aesculin, gelatin, casein and starch are hydrolysed but agar, DNA, Tweens 20, 40 and 80, urea, cellulose (CM-cellulose and filter paper) and chitin are not. Acid is produced from cellobiose, D-glucose and maltose, but not from L-arabinose, D-galactose, D-fructose, D-lactose, melibiose, raffinose, L-rhamnose, sucrose, xylose, N-acetylglucosamine, glycerol or mannitol. Utilizes inositol, mannitol and sorbitol, but not L-arabinose, D-lactose, mannose, sucrose, N-acetylglucosamine, adipate, caprate, citrate, gluconate, malate, malonate and phenylacetate. According to the API ZYM gallery, esterase (C4) (weakly), esterase lipase (C8), leucine arylamidase, cystine arylamidase, valine arylamidase, alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but lipase (C14), trypsin, α-chymotrypsin, α- and β-galactosidases, β-glucuronidase, α- and β-glucosidases, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. No flexirubins are formed. Nitrate is not reduced. Hydrogen sulphide is produced, but indole and acetoin are not. Brown pigment is not formed on tyrosine medium. Susceptible to ampicillin, benzylpenicillin, carbenicillin, cefalexin, chloramphenicol, erythromycin, lincomycin, ofloxacin, oleandomycin, rifampicin and vancomycin; and resistant to cefazolin, doxycycline, gentamicin, kanamycin, neomycin, nalidixic acid, oxacillin, polymyxin B, streptomycin and tetracycline. The predominant fatty acids are iso-C_{15:0}, iso-C_{15:1}, anteiso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{16:0} 3-OH, C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. The DNA G+C content of the type strain is 35.3 mol%.

The type strain, KMM 6019^T (=KCTC 22997^T=LMG 22568^T), was isolated from shallow seawater collected from Amursky Bay, the East Sea, also known as the Sea of Japan.

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