Winogradskyella echinorum sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from the sea urchin *Strongylocentrotus intermedius*

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The taxonomic position of a novel marine, yellow-pigmented bacterium, designated strain KMM 6211^T, was examined by using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain KMM 6211^T is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. The closest relative of strain KMM 6211^T was *Winogradskyella eximia* KMM 3944^T, the sequence similarity being 97.1 %. The DNA G+C content of KMM 6211^T was 33.6 mol%. The strain was motile by gliding and grew with 1–6 % NaCl and at 4–37 °C. Aesculin, casein and gelatin were hydrolysed, but agar, starch, DNA and chitin were not degraded. On the basis of phylogenetic data and phenotypic differences between the isolate and recognized *Winogradskyella* species, strain KMM 6211^T represents a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella echinorum* sp. nov. is proposed. The type strain is KMM 6211^T (=KCTC 22026^T=LMG 24757^T).

The genus *Winogradskyella* was created to accommodate heterotrophic, aerobic, yellow-pigmented, Gram-negative and motile (gliding) bacteria (Nedashkovskaya *et al.*, 2005). At the time of writing, the genus comprises four recognized species: *Winogradskyella epiphytica*, *Winogradskyella eximia*, *Winogradskyella poriferorum* and *Winogradskyella thalassocola* (Lau *et al.*, 2005; Nedashkovskaya *et al.*, 2005). The type strains of the species in the genus *Winogradskyella* were isolated from the green alga *Acrosiphonia sonderi*, from the brown algae *Chorda filum* and *Laminaria japonica* (collected in the East Sea, also known as the Sea of Japan) and from the sponge *Lissodendoryx isodictyalis* (collected in the Bahamas). Representatives of the genus *Winogradskyella* were also found during a study of bacterial communities of Arctic pack ice, from North Sea green alga (*Enteromorpha*) and from coastal surface water (Alonso *et al.*, 2007; Brinkmeyer *et al.*, 2003; Patel *et al.*, 2003).

The aim of this study was to determine the precise taxonomic position of a sea urchin-associated micro-organism, designated strain KMM 6211^T, by using a polyphasic approach. The results of a phylogenetic analysis based on 16S rRNA gene sequences revealed that the novel isolate belonged to the family *Flavobacteriaceae* and occupied a distinct lineage within the genus *Winogradskyella*.

Strain KMM 6211^T was isolated from a sea urchin (*Strongylocentrotus intermedius*) collected in Troitsa Bay in the Gulf of Peter the Great of the East Sea (also known as the Sea of Japan). For strain isolation, 0.1 ml homogenates of sea-urchin tissues were transferred onto plates of marine agar 2216 (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 2216 (Difco) supplemented with 20 % (v/v) glycerol.

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene were performed according to procedures described previously (Vancanneyt *et al.*, 2004). The sequence data obtained were aligned with those of representative members of selected genera belonging to the family *Flavobacteriaceae* by using PHYDIT, version 3.2

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 6211^T is EU727254.

(http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred by using suitable programs in the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated by using the model of Jukes & Cantor (1969) and the tree was constructed with the neighbour-joining (Saitou & Nei, 1987) method. Phylogenetic trees were also constructed by using the maximum-likelihood and maximum-parsimony methods (Felsenstein, 1993). Bootstrap analysis was performed with 100 resampled datasets, using the SEQBOOT and CONSENSE programs of the PHYLIP package.

A phylogenetic analysis of almost-complete 16S rRNA gene sequences indicated that strain KMM 6211^T was a member of the family *Flavobacteriaceae* and formed a distinct branch within the genus *Winogradskyella* (Fig. 1). The levels of 16S rRNA gene sequence similarity between strain KMM 6211^T and the type strains of species in the genus *Winogradskyella* ranged from 95.2 to 97.1 %. Although *W. eximia* KMM 3944^T was the closest relative of the novel isolate (97.1 % sequence similarity), the tree topology showed that strain KMM 6211^T clustered with *W. poriferorum* UST030701-295^T (Fig. 1).

DNA was isolated by following the method of Marmur (1961) and the DNA G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The DNA G+C content of KMM 6211^T was 33.6 mol%.

To determine whole-cell fatty acid and polar lipid profiles, strain KMM 6211^T was grown at 28 °C for 48 h on marine agar 2216. The polar lipids and fatty acid methyl esters were extracted and analysed as described previously (Nedashkovskaya *et al.*, 2006).

Phosphatidylethanolamine was the only phospholipid identified. The predominant cellular fatty acids of strain KMM 6211^T were straight-chain unsaturated, branched-chain unsaturated and saturated, namely iso-$C_{15:1}$, iso-$C_{15:0}$, summed feature 3 (consisting of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{15:0}$, iso-$C_{15:0}$ 3-OH and iso-$C_{17:0}$ 3-OH. These values are consistent with those reported for recognized members of the genus *Winogradskyella* (Lau *et al.*, 2005; Nedashkovskaya *et al.*, 2005).

The physiological, morphological and biochemical characteristics of strain KMM 6211^T were tested as described previously (Nedashkovskaya *et al.*, 2003, 2004). API 20NE and API ZYM galleries (bioMérieux) were also used for studying the phenotypic features of the strain; they were performed according to the manufacturer's instructions, except that the galleries were incubated at 28 °C.

The main physiological and biochemical characteristics are given in the species description and in Table 1. Phenotypic similarities between KMM 6211^T and *Winogradskyella* species support the classification of the strain within the genus *Winogradskyella*. Like other *Winogradskyella* species, the novel isolate demonstrates oxidase, catalase, alkaline phosphatase and gelatinase activities, requires Na^+ ions for growth and moves by gliding (Table 1). However, in contrast with the recognized *Winogradskyella* species, strain KMM 6211^T shows β-galactosidase activity and lacks esterase (Tween 40) activity. Strain KMM 6211^T clearly differs from its closest phylogenetic relative, *W. eximia* KMM 3944^T, in terms of hydrolysis of agar and starch, growth at 37 °C, acid production from D-glucose, maltose, sucrose and mannitol and utilization of sucrose and mannitol (Table 1). The traits that serve to distinguish strain KMM 6211^T from the recognized *Winogradskyella* species are shown in Table 1.

Therefore, the data obtained in this polyphasic analysis confirm that strain KMM 6211^T represents a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella echinorum* sp. nov. is proposed.

Description of *Winogradskyella echinorum* sp. nov.

Winogradskyella echinorum (e.chi.no′rum. L. masc. n. *echinus* sea urchin; N.L. gen. pl. n. *echinorum* of echini, sea urchins, referring to the isolation of the type strain from a sea urchin).

Cells are Gram-negative rods that are motile by gliding, 0.4–0.5 μm wide and 1.2–2.7 μm long. On marine agar 2216, colonies are 1–2 mm in diameter, circular, shiny with entire edges and are pigmented bright yellow. Growth occurs at 4–37 °C and with 1–6 % NaCl. Produces oxidase,

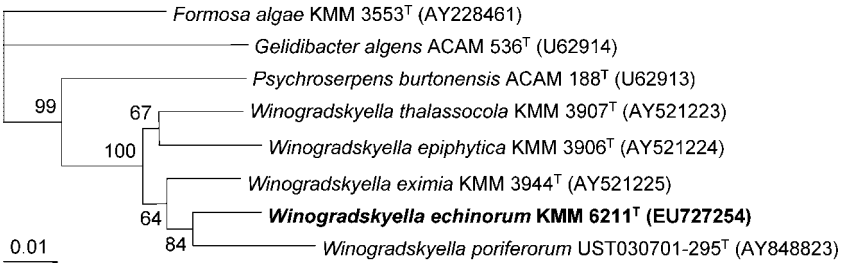

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain KMM 6211^T and type strains of species of the genus *Winogradskyella*. The tree was constructed by using Jukes–Cantor evolutionary distances. Numbers at nodes indicate bootstrap percentages (based on 100 resampled datasets). Exactly the same topology was recovered in the maximum-likelihood and maximum-parsimony trees (data not shown). Bar, 0.01 substitutions per nucleotide position.

Table 1. Phenotypic features of KMM 6211^T and strains of the recognized *Winogradskyella* species

Taxa: 1, KMM 6211^T; 2, *W. thalassocola* KMM 3907^T; 3, *W. epiphytica* KMM 3906^T; 4, *W. eximia* KMM 3944^T; 5, *W. poriferorum* UST030701-295^T. Data are from Lau *et al.* (2005), Nedashkovskaya *et al.* (2005) 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 benzyl penicillin, 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, inositol, sorbitol, malonate and citrate. ND, Data not available.

Characteristic	1	2	3	4	5
β-Galactosidase activity	+	−	−	−	−
Degradation of:					
Agar	−	+	+	+	−
Casein	+	−	−	+	−
Starch	−	−	−	+	−
Tween 20	−	−	+	+	+
Tween 40	−	+	+	+	+
Tween 80	−	−	+	−	+
DNA	+	−	+	−	+
Growth at/with:					
37 °C	+	−	+	−	+
44 °C	−	−	−	−	+
8 % NaCl	−	+	+	−	−
Acid formation from:					
D-Glucose	−	+	−	+	−
Maltose	−	+	−	+	−
Cellobiose	−	+	−	−	−
Sucrose	−	−	−	+	−
Mannitol	−	−	−	+	−
Utilization of:					
D-Glucose	+	+	−	+	−
D-Mannose	+	+	−	+	−
Sucrose	−	−	−	+	−
Mannitol	−	−	−	+	−
Susceptibility to:					
Ampicillin	−	−	+	−	+
Oleandomycin	+	+	+	−	ND
Tetracycline	−	−	+	+	+
DNA G+C content (mol%)	33.6	34.6	35.2	36.1	32.8

catalase, β-galactosidase and alkaline phosphatase. Degrades aesculin (weakly), gelatin, casein and DNA. Does not hydrolyse agar, starch, Tweens 20, 40 or 80, cellulose (CM-cellulose or filter paper) or chitin. Does not produce acid from L-arabinose, cellobiose, D-galactose, D-glucose, D-fructose, D-lactose, maltose, melibiose, raffinose, L-rhamnose, sucrose, D-xylose, *N*-acetylglucosamine, glycerol, inositol, sorbitol or mannitol. Utilizes D-glucose and D-mannose, but not L-arabinose, D-lactose, maltose, sucrose, *N*-acetylglucosamine, adipate, caprate, citrate, gluconate, malate, phenyl acetate, inositol, mannitol or sorbitol. In the API ZYM gallery, esterase C4, esterase lipase C8, leucine, cystine and valine arylamidases, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but lipase C14, trypsin, α-galactosidase, β-glucuronidase, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Nitrate is not reduced. H_2S, indole and acetoin (Voges–Proskauer reaction) are not produced. Susceptible to carbenicillin, chloramphenicol, erythromycin, lincomycin and oleandomycin, but resistant to ampicillin, benzyl penicillin, doxycycline, gentamicin, kanamycin, neomycin, polymyxin B, streptomycin and tetracycline. Phosphatidylethanolamine is the only phospholipid detected. The predominant fatty acids are iso-$C_{15:1}$, iso-$C_{15:0}$, summed feature 3 (consisting of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{15:0}$, iso-$C_{15:0}$ 3-OH and iso-$C_{17:0}$ 3-OH. The DNA G+C content of the type strain is 33.6 mol%.

The type strain, KMM 6211^T (=KCTC 22026^T=LMG 24757^T), was isolated at Troitsa Bay in the East Sea (also known as the Sea of Japan) from the sea urchin *Strongylocentrotus intermedius*.

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