Winogradskyella exilis sp. nov., isolated from the starfish Stellaster equestris, and emended description of the genus Winogradskyella

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A pale-yellowish-pigmented strain, 022-2-26T, was isolated from a starfish, Stellaster equestris. Cells of strain 022-2-26T were Gram-negative short rods that were chemo-organotrophic, alkalitolerant and mesophilic. The predominant menaquinone was MK-6. The major cellular fatty acids were iso-C15:0, iso-C15:1,C15:0, iso-C15:0:2-OH and iso-C17:0:3-OH (together representing 87 % of the total fatty acids). The DNA G+C content was 30.1 mol%. A 16S rRNA gene sequence of the isolate was determined and phylogenetic analyses revealed that strain 022-2-26T formed a robust clade (neighbour-joining algorithm with a bootstrap value of 95 % and parsimony and maximum-likelihood algorithms) with type strains of species in the genus Winogradskyella. The closest phylogenetic neighbour of strain 022-2-26T was Winogradskyella poriferorum UST030701-295T (96 % 16S rRNA gene sequence similarity; 59 differences between sequences). On the basis of the phenotypic and chemotaxonomic characteristics and the phylogenetic evidence, it is proposed that strain 022-2-26T represents a novel species, Winogradskyella exilis sp. nov. The type strain is 022-2-26T (=KMM 6013T=CIP 109976T).

The genus Winogradskyella was created within the family Flavobacteriaceae in 2005 to accommodate three species, Winogradskyella thalassocola, W. epiphytica and W. eximia, marine bacteria respectively isolated from the green alga Acrosiphonia sonderi and the brown algae Chorda filum and Laminaria japonica (Nedashkovskaya et al., 2005). A fourth species was described shortly afterwards: Winogradskyella poriferorum, isolated from a sponge (Lau et al., 2005). We report here the polyphasic characterization of a novel bacterium isolated from the starfish Stellaster equestris.

The starfish was collected with dragging equipment in October 1998 at a depth of 100 m (30 % salinity, 15 °C) in the South China Sea (26°28.3′ N 122°29.0′ E). The starfish was pre-rinsed in sterilized seawater and a piece (about 3 g) of tegument tissue was removed aseptically. Strain 022-2-26T was isolated from the tissue homogenates by plating samples (0.1 ml) on agar plates of marine agar 2216 (MA; Oxoid) and on medium B. Medium B contained (w/v) 0.2 % Bacto peptone (Difco), 0.2 % casein hydrolysate (Merck), 0.2 % Bacto yeast extract (Oxoid), 0.1 % glucose, 0.02 % KH2PO4, 0.005 % MgSO4, 7H2O and 1.5 % Bacto agar (Oxoid) in 50 % (v/v) natural seawater and 50 % (v/v) distilled water at pH 7.5–7.8, as described elsewhere (Ivanova et al., 1996).

The phenotypic properties used for the characterization of Flavobacterium species were determined by using standard procedures (Smibert & Krieg, 1994) and as described elsewhere (McMeekin et al., 1971; Ivanova et al., 1996, 1998, 2005; Bernardet et al., 2002). Strain 022-2-26T was cultured at 22–24 °C. To test for spreading growth and gliding motility, strain 022-2-26T was grown on medium B with the peptone content reduced to 0.02 % (0.2 g l−1). Gliding motility was verified by using phase-contrast microscopy and was described as positive if the bacteria grew in a straight line extending for more than 100 μm.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 022-2-26T is FJ595484.

A table of fatty acid compositions and a 16S rRNA gene sequence-based consensus tree are available as supplementary material with the online version of this paper.
microscopy (Eclipse TE-DH, 100 W; Nikon) of hanging-drop preparations. The bathochromic shift test with 20\% (w/v) KOH was performed to detect flexirubin pigments (Fautz & Reichenbach, 1980). The following physiological and biochemical properties of strain 022-2-26^T were examined: oxidation/fermentation of glucose (Hugh & Leifson, 1953), Gram stain, reduction of nitrate and nitrite, liquefaction of gelatin, activity of catalase (with 5\% H_2O_2), oxidase (Kövacs, 1956), arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase, production of poly-β-hydroxybutyrate, acetoin (Voges-Proskauer test), indole and H_2S, requirement for sodium ions (0, 1, 3, 6, 8, 10, 12, 15\%, w/v; NaCl) in nutrient broth (Oxoid) and liquefaction of agar, alginate, casein, chitin, DNA, starch and Tween 80. The temperature range for growth was examined on MA at 4, 10, 30, 35, 37 and 42 °C. Other physiological and biochemical tests were performed with the API 20E, API 50 and API ZYM systems (bioMérieux). Susceptibility to antibiotics was tested by the diffusion plate technique, using solid medium B and discs impregnated with the following antibiotics (µg per disc unless otherwise indicated): ampicillin (10), benzylpenicillin (10 U), carbencillin (100), cephalaxin (30), cephalzin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (15), nalidixic acid (30), neomycin (30), ofloxacin (5), oleandomycin (15), oxacillin (10), polymyxin (300 U), rifampicin (5), streptomycin (30) and tetracycline (30). The physiological and biochemical characteristics are presented in the species description and in Table 1.

For the extraction and characterization of carotenoid pigments and menaquinones, phospholipids and fatty acids, cell biomass of strain 022-2-26^T was harvested from MA (five plates for carotenoid pigment analysis) after incubation at 28 °C for 48 h as described previously (Ivanova et al., 2005). Pigment extraction and characterization was performed as described previously (Ivanova et al., 2005). The isoprenoid quinone composition was characterized by HPLC-MS (model 1200L; Varian) using reversed-phase Omnisphere 3 C18 column (20 cm × 0.25 mm). The column temperature was programmed for a hold at 170 °C for 1 min, an increase to 240 °C at 2 °C min^{-1} and a hold at 240 °C for 20 min. The temperature of the detector and injector were 250 °C. The results of the fatty acid analysis were presented in the species description and Supplementary Table S1 (available in IJSEM Online). Strain 022-2-26^T contained MK-6, which is the characteristic respiratory quinone for the family Flavobacteriaceae, and high levels of branched and hydroxy C_{15} and C_{17} fatty acids (representing 92.9\% of the total fatty acid content), which are typical fatty acids for the family Flavobacteriaceae (Bowman et al., 1998).

Table 1. Characteristics that differentiate strain 022-2-26^T from the type strains of the most closely related Winogradskyella species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td><strong>Ranges for growth</strong></td>
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<tr>
<td>Temperature (°C)</td>
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<td>12–44</td>
<td>4–37</td>
<td>4–33</td>
<td>4–33</td>
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<tr>
<td>NaCl (%, w/v)</td>
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<td>1–4</td>
<td>1–8</td>
<td>1–5</td>
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<td>Urease</td>
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<td>Denitrification</td>
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<td>Agar</td>
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<td>–</td>
<td>+</td>
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<tr>
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<td>+</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>30.2</td>
<td>32.8</td>
<td>35.2</td>
<td>36.1</td>
<td>34.6</td>
</tr>
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</table>

Genomic DNA was extracted from strain 022-2-26^T following the method of Marmur (1961) and the G+C content of the DNA was determined by the thermal denaturation method of Marmur & Doty (1962). The G+C content of the DNA was 30.1 ± 0.2 mol\%. The 16S
rRNA gene was amplified and sequenced at the laboratories of the Australian Genome Research Facility, Brisbane, Australia. Briefly, the PCR primers corresponded to Escherichia coli positions 5 and 1540. The amplification products were purified using Microcon 100 molecular-mass cut-off membranes (Millipore) and checked for quality and quantity on separation on agarose gel. Cycle sequencing of the amplification products was performed using AmpliTaq ES DNA polymerase and rhodamine dye terminators and the sequencing reaction products were analysed with an ABI Prism 377 DNA Sequencer (Applied Biosystems).

The 16S rRNA gene sequence of strain 022-2-26\textsuperscript{T} was compared against a database of cultured species (http://bioinfo.unice.fr/blast) and type strains (EzTaxon; http://147.47.212.35:8080) in order to retrieve the 110 most similar sequences. A multiple sequence alignment was performed using CLUSTAL W version 2 (Larkin et al., 2007) and checked manually with SeaView (Galtier et al., 1996). Domains common to all sequences were used to derive a preliminary phylogenetic tree (not shown) using the most recent version of SeaView (version 4; Gouy et al., 2010) and the BioNJ neighbour-joining algorithm (Gascuel, 1997). This tree showed that strain 022-2-26\textsuperscript{T} was most closely related to strains in the genus Winogradskyella. According to the preliminary tree, the 44 sequences most closely related to strain 022-2-26\textsuperscript{T} were selected and the alignment was checked again. Positions containing insertions or deletions were excluded. A full phylogenetic analysis was undertaken using the BioNJ, parsimony and maximum-likelihood algorithms, according to Gascuel (1997), PHYLIP version 3.52 and PhyML version 3.0.1 (Guindon & Gascuel, 2003), respectively. For the neighbour-joining analysis, a distance matrix was calculated using Kimura’s two-parameter correction and bootstraps were performed using CLUSTAL W version 2 (Larkin et al., 2007) and PhyML version 3.0.1 (Guindon & Gascuel, 2003), respectively. For the neighbour-joining analysis, a distance matrix was calculated using Kimura’s two-parameter correction and bootstraps were performed using 1000 replications. Phylogenetic trees were drawn using TreeDyn (Chevenet et al., 2006) or SeaView. A consensus phylogenetic tree is shown in Supplementary Fig. S1. Notably, when using similarity values only, the closest neighbour to strain 022-2-26\textsuperscript{T} was W. poriferorum UST030701-295\textsuperscript{T}, with which the isolate showed a similarity of about 96 % (59 differences between sequences) and formed a robust cluster with all algorithms and a bootstrap value of 82 % (Fig. 1). Strain 022-2-26\textsuperscript{T} was also robustly included in the clade formed by the type strains of Winogradskyella species (all methods, bootstrap value of 95 %). Thus, the phylogenetic analyses provide strong evidence that strain 022-2-26\textsuperscript{T} represents a novel species of the genus Winogradskyella.

Strain 022-2-26\textsuperscript{T} can be differentiated easily from the type strains of previously described species of the genus Winogradskyella. For example, strain 022-2-26\textsuperscript{T} does not have a requirement for NaCl for growth and it is negative for oxidase and positive for denitrification. However, the fatty acid profile of strain 022-2-26\textsuperscript{T} is characteristic of the genus Winogradskyella, members of which have levels of iso-C\textsubscript{15:0} 2-\text{OH} and iso-C\textsubscript{17:0} 3-\text{OH} ranging from 4.2 to 10.2 % and distinct proportions of iso-C\textsubscript{15:0} and iso-C\textsubscript{16:1} as the major fatty acids. Consequently, we consider that strain 022-2-26\textsuperscript{T} represents a novel species in the genus Winogradskyella, for which the name Winogradskyella exilis sp. nov. is proposed.

Emended description of the genus Winogradskyella Nedashkovskaya et al. 2005

The description is as given by Nedashkovskaya et al. (2005) with the following amendments. Some strains are oxidase-negative and able to reduce nitrate. Some strains do not require NaCl for growth.

Description of Winogradskyella exilis sp. nov.

Winogradskyella exilis (e.x’i’ls. L. fem. adj. exilis small, thin, slender).

Rod-shaped cells with slightly irregular sides and pointed ends, about 0.8–1.8 \(\mu\)m long and 0.4–0.9 \(\mu\)m wide. Gram-negative. No endospores or resting stages. Exhibits gliding motility. Does not accumulate poly-\(\beta\)-hydroxybutyrate as an intracellular reserve product or have an arginine dihydrolase system. Aerobic and facultatively anaerobic. Anaerobic growth occurs by fermentation of D-glucose or anaerobic respiration of nitrate. Chemo-organotrophic. Oxidase-negative and catalase-positive. Colonies are light yellow, circular, 1–3 mm in diameter and low convex on solid media with a high content of nutrient components. Produces carotenoid pigments with absorbance peaks at 455 and 480 nm. Does not produce flexirubin pigments.

![Fig. 1. Unrooted neighbour-joining phylogenetic tree showing the position of strain 022-2-26\textsuperscript{T} among its closest phylogenetic neighbours, based on 16S rRNA gene sequences. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in trees constructed with both the maximum-likelihood and parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.](http://ijs.sgmjournals.org/10.1099/ijs.0.005341-0)
Grows optimally at 23 °C; no growth at 4 or 37 °C. Grows at pH 6.0–10.0 (optimum pH 8.0–8.5) and with 0–6 % NaCl. Weakly hydrolyses starch and gelatin. Does not decompose agar or chitin. Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and valine arylamidase. With the API 50 system, utilizes only potassium 5-ketogluconate. Susceptible to (μg per disc unless otherwise indicated) ampicillin (10), benzylpenicillin (10 U), carbenicillin (100), cephalin (30), cephalin (30), chloramphenicol (30), lincomycin (15), nalidixic acid (30), ofloxacin (5), oleanomycin (15), rifampicin (5) and tetracycline (30), but not to erythromycin (15), gentamicin (10), kanamycin (30), neomycin (30), oxacillin (10), polymyxin (300 U) or streptomycin (30). The main cellular fatty acids are iso-C15:0, iso-C15:0 2-OH and iso-C17:0 3-OH. Phosphatidylethanolamine is the only phospholipid detected; aminolipids are not detected. The major isoprenoid quinone is MK-6. The DNA G+C content of the type strain is 30.0 ± 0.2 mol%.

The type strain is 022-2-26 T (=KMM 6013 T =CIP 109976 T), isolated from the starfish *Stellaster equestris*.

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