Winogradskyella poriferorum sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a sponge in the Bahamas

Stanley C. K. Lau,[1] Mandy M. Y. Tsoi,[1] Xiancui Li,[1] Ioulia Plakhotnikova,[1] Sergey Dobretsov,[1] Ken W. K. Lau,[1] Madeline Wu,[1] Po-Keung Wong,[2] Joseph R. Pawlik[3] and Pei-Yuan Qian[1]

Correspondence
Pei-Yuan Qian
boqianpy@ust.hk

[1]Coastal Marine Laboratory/Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, People's Republic of China

[2]Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, People's Republic of China

[3]Center for Marine Science, University of North Carolina at Wilmington, USA

A Gram-negative, rod-shaped bacterium (designated strain UST030701-295^T) with fast gliding motility was isolated from the surface of the sponge *Lissodendoryx isodictyalis* in the Bahamas. Colonies of UST030701-295^T were yellow in colour, 2–4 mm in diameter, convex with a smooth surface and entire margins. UST030701-295^T was heterotrophic, strictly aerobic and required NaCl for growth (1·0–4·0 %). Growth was observed at pH 6·0–10·0 and at 12–44 °C. Phylogenetic analysis of the 16S rRNA gene sequence placed UST030701-295^T within the genus *Winogradskyella* of the family *Flavobacteriaceae*, sharing 94·7–95·8 % similarity with the three recognized members of the genus. The G+C content of the DNA was 32·8 mol% and the predominant fatty acids were iso-$C_{15:1}$, iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$ 3-OH, iso-$C_{16:0}$ 3-OH, $C_{16:1}\omega7$ and iso-$C_{17:0}$ 3-OH (together representing 75·4 % of the total); these data supported the affiliation of UST030701-295^T to the genus *Winogradskyella*. UST030701-295^T differed from the three recognized species of *Winogradskyella* in 7–17 traits. Molecular evidence together with phenotypic characteristics suggests that UST030701-295^T represents a novel species within the genus *Winogradskyella*, for which the name *Winogradskyella poriferorum* sp. nov. is proposed. The type strain is UST030701-295^T (=NRRL B-41101^T=JCM 12885^T).

The *Cytophaga–Flavobacterium–Bacteroides* group, comprising the families *Bacteroidaceae*, *Cytophagaceae*, *Cryomorphaceae*, *Flavobacteriaceae*, *Sphingobacteriaceae* and *Spirosomaceae*, is a main phyletic line within the domain *Bacteria* (Bernardet *et al.*, 2002; Bowman *et al.*, 2003). Many members of the *Flavobacteriaceae* have been isolated from the surfaces of marine algae (Nedashkovskaya *et al.*, 2005). *Winogradskyella* is a recently established genus within the family *Flavobacteriaceae* (Nedashkovskaya *et al.*, 2005). The three recognized members of *Winogradskyella* were isolated from algal frond surfaces in the Sea of Japan (Nedashkovskaya *et al.*, 2005). In this study, we describe a novel member of the genus isolated from the surface of a sponge in tropical water.

During the characterization of bacteria isolated from the surface of the sponge *Lissodendoryx isodictyalis* in the Bahamas, strain UST030701-295^T was isolated on an agar medium consisting of 5 g peptone l^{-1}, 3 g yeast extract l^{-1} and 0·22-μm-filtered seawater (hereafter marine agar) after 48 h of incubation at 30 °C. Unless otherwise specified, all characteristics described are based on cultures grown on marine agar under these conditions. Cells of strain UST030701-295^T appeared as yellow, convex, circular colonies (2–4 mm in diameter) with entire margins and a smooth surface. No diffusible pigment was observed.

The nearly complete 16S rRNA gene sequence of UST030701-295^T (1441 bp) was resolved on a MegaBACE capillary genetic analyser using a dye terminator method according to the manufacturer's protocol. Primers used in the sequencing reactions are given in Supplementary

Published online ahead of print on 4 March 2005 as DOI 10.1099/ijs.0.63661-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST030701-295^T is AY848823.

A scanning electron micrograph of cells of strain UST030701-295^T and tables detailing the primers used for construction of the 16S rRNA gene sequences and results of carbohydrate metabolism tests are available as supplementary material in IJSEM Online.

Table S1 available in IJSEM Online. Fragments of DNA sequence obtained from individual primers with at least six replicates each were assembled using the Sequencher software package (Gene Codes). Comparison of the 16S rRNA gene sequence of strain UST030701-295^T to those available from GenBank revealed that UST030701-295^T represented a member of the family *Flavobacteriaceae*. Its closest relatives were *Winogradskyella epiphytica* KMM 3906^T (95·8 % 16S rRNA gene sequence similarity), *Winogradskyella eximia* KMM 3944^T (94·7 %) and *Winogradskyella thalassocola* KMM 3907^T (94·7 %) (Nedashkovskaya *et al.*, 2005). A neighbour-joining phylogenetic tree (Fig. 1) constructed using the ARB software package (Ludwig *et al.*, 2004) indicated that UST030701-295^T and the three recognized species of *Winogradskyella* belonged to the same clade. Within this clade, UST030701-295^T and *W. eximia* formed a separate branch, which clustered robustly (99 % bootstrap support, 500 replicates) with the branch formed by *W. epiphytica* and *W. thalassocola*. Trees based on maximum-parsimony and maximum-likelihood methods showed the same topology. The results of phylogenetic analysis suggest that strain UST030701-295^T represents a novel species within the genus *Winogradskyella*.

The G+C content of the DNA of UST030701-295^T, as determined by using an HPLC method (Mesbah *et al.*, 1989), was 32·8±0·7 mol%. This value is similar to those described for *W. epiphytica* (35·2 mol%), *W. eximia* (36·1 mol%) and *W. thalassocola* (34·6 mol%). The predominant cellular fatty acids of UST030701-295^T were iso-$C_{15:1}$, iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$ 3-OH, iso-$C_{16:0}$ 3-OH, $C_{16:1}\omega7$ and iso-$C_{17:0}$ 3-OH (together representing 75·4 % of the total) as determined using the Sherlock Microbial Identification System according to the manufacturer's protocol. This fatty acid profile is similar to that of the three recognized members of *Winogradskyella* (Table 1). MK-6 is the only respiratory quinone present, as determined using an HPLC method according to Collins (1994). Menaquinones extracted from *Cytophaga lytica* (Nakagawa & Yamasato, 1993) and *Sphingobacterium heparinum* (Steyn *et al.*, 1998) served as references for MK-6 and MK-7, respectively.

The phenotypic characteristics of UST030701-295^T are given under the species description below. Anaerobic growth was examined in the Oxoid Anaerobic System. Requirement for NaCl was tested in a medium containing 5 g $MgCl_2\,l^{-1}$, 2 g $MgSO_4\,l^{-1}$, 0·5 g $CaCl_2\,l^{-1}$, 1 g $KCl\,l^{-1}$, 5 g peptone l^{-1} and various amounts of NaCl adjusted to pH 7·5 using KOH (Isnansetyo & Kamei, 2003). Cell morphology was examined using scanning electron microscopy (JEOL 7600F) according to the procedures detailed in Neu *et al.* (2001) (see Supplementary Fig. S1 in IJSEM Online). Reaction to Gram-stain was determined using light microscopy according to the method of Smibert & Krieg (1994). Gliding motility was determined using phase-contrast light microscopy as described by Bowman (2000). Susceptibility to antibiotics was tested using the method of Acar (1980). Oxidase and catalase activities and the degradation of agar, DNA and starch were tested according to the methods of Smibert & Krieg (1994). Flexirubin pigment production and cellulose hydrolysis were determined as described by Bowman (2000). Casein hydrolysis

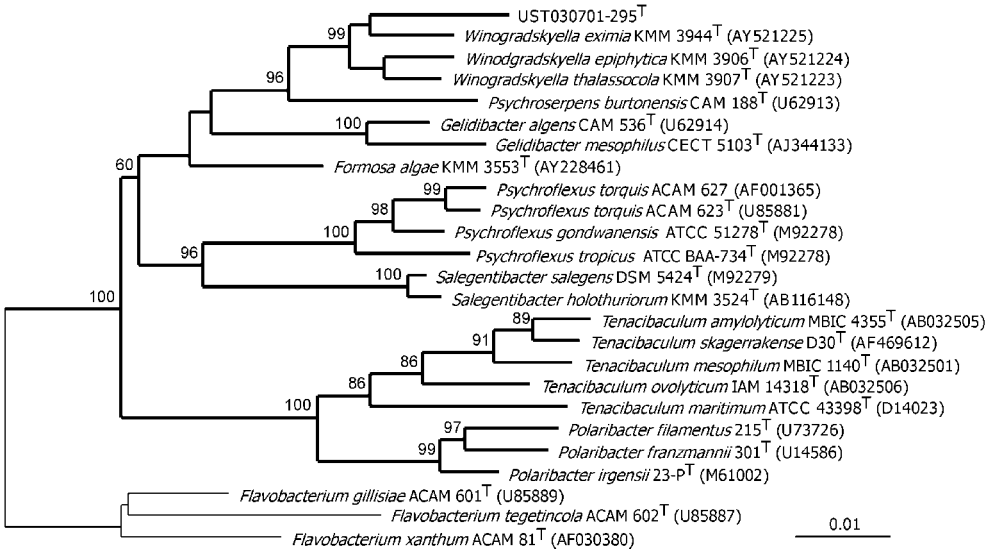

Fig. 1. Unrooted neighbour-joining dendrogram showing the estimated phylogenetic relationships among UST030701-295^T and related species on the basis of 16S rRNA gene sequences. Strains belonging to the genus *Flavobacterium* served as outgroups. Lines in bold indicate branches also found in maximum-likelihood and maximum-parsimony trees. Bootstrap values of >50 % (500 replicates) are indicated at the nodes. The GenBank accession number for each reference strain is shown in parentheses. Bar, 1 nucleotide substitution per 100 nucleotides.

Table 1. Cellular fatty acid profiles of UST030701-295^T and previously described members of the genus *Winogradskyella*

Taxa: 1, UST030701-295^T; 2, *W. epiphytica*; 3, *W. eximia*; 4, *W. thalassocola*. Values given for UST030701-295^T are mean percentages ± SD ($n=3$) of the total fatty acids. Data for *W. epiphytica*, *W. eximia* and *W. thalassocola* are taken from Nedashkovskaya *et al.* (2005).

Fatty acid	1	2	3	4
iso-$C_{13:0}$	2·6±0·1	–	–	–
iso-$C_{14:0}$	1·2±0·1	4·5	1·4	2·6
iso-$C_{14:0}$ 3-OH	0·5±0·1	1·6	–	0·9
iso-$C_{14:1}$	2·0±0·3	1·4	–	–
$C_{15:0}$	–	1·2	6·7	7·9
anteiso-$C_{15:0}$	–	15·9	7·0	4·9
iso-$C_{15:0}$	12·6±1·1	6·7	25·6	8·7
iso-$C_{15:0}$ 3-OH	9·8±1·0	2·9	2·6	11·9
$C_{15:0}$ 2-OH	3·1±0·5	3·3	1·0	1·8
$C_{15:0}$ 3-OH	2·4±0·7	–	–	2·5
$C_{15:1}\omega6$	–	–	–	6·5
anteiso-$C_{15:1}$	1·5±0·2	6·3	1·4	1·6
iso-$C_{15:1}$	20·9±0·6	8·1	10·4	11·4
iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7$*	9·8±0·8	5·1	6·1	4·2
$C_{16:0}$ 10-methyl	–	–	6·3	–
$C_{16:0}$ 3-OH	1·3±0·1	–	–	1·0
iso-$C_{16:0}$	0·5±0·2	3·7	5·7	0·8
iso-$C_{16:0}$ 3-OH	11·43±0·1	17·1	3·2	18·1
iso-$C_{16:1}$	0·8±0·3	3·5	4·7	2·7
$C_{17:0}$ 2-OH	0·3±0·0	5·2	1·0	0·8
$C_{17:}$0 cyclo	–	–	2·4	–
iso-$C_{17:0}$ 3-OH	10·2±0·7	7·3	6·7	5·4
anteiso-$C_{17:1}$	–	–	2·3	–
iso-$C_{17:1}\omega9$	–	1·1	–	0·6
$C_{17:1}\omega6$	0·2±0·1	1·9	–	0·9
Unknown	3·9±0·6	3·7	5·6	4·8

*Appeared as a summed feature.

Table 2. Characteristics used to differentiate UST030701-295^T from the three recognized members of the genus *Winogradskyella*

Taxa: 1, UST030701-295^T; 2, *W. epiphytica*; 3, *W. eximia*; 4, *W. thalassocola*. Data for *W. epiphytica*, *W. eximia* and *W. thalassocola* are taken from Nedashkovskaya *et al.* (2005). All are susceptible to kanamycin. All are positive for degradation of gelatin and Tween 40 and for alkaline phosphatase, catalase and oxidase activities. All are negative for nitrate reduction, β-galactosidase and urease activities, cellulose and chitin degradation, flexirubin and indole production and citrate, D-adonitol, L-arabinose, dulcitol, D-galactose, inositol, D-lactose, D-melibiose, L-rhamnose, D-sorbitol, D-xylose and L-xylose utilization.

Characteristic	1	2	3	4
Growth in/at:				
NaCl (%)	1·0–4·0	1·0–8·0	1·0–5·0	1·0–8·0
Temperature (°C)	12·0–44·0	4·0–37·0	4·0–33·0	4·0–33·0
G+C content (mol%)	32·8	35·2	36·1	34·6
Susceptibility to:				
Ampicillin	+	+	–	–
Benzylpenicillin	+	–	–	–
Streptomycin	+	–	–	–
Tetracycline	+	+	–	–
Production of acetoin	+	–	–	–
Degradation of:				
Agar	–	+	+	+
Casein	–	–	+	–
DNA	+	+	–	–
Starch	–	–	+	–
Tween 20	+	+	+	–
Tween 80	+	+	–	–
Metabolism of:				
D-Cellobiose	–	–	–	+
D-Glucose	–	–	+	+
D-Maltose	–	–	+	+
D-Mannitol	–	–	+	–
D-Mannose	–	–	+	+
Sucrose	–	–	+	+

was determined according to the method of Norris *et al.* (1985); hydrolysis of chitin and Tweens 20, 40 and 80 was determined according to Baumann & Baumann (1981). Substrate utilization patterns and other enzymic activities were tested using the commercial systems API 20E, API 20NE, API 50CH and API ZYM (bioMérieux). Cells for inoculation to the API systems were suspended in a sterile solution of seawater mixture at 22 ‰ salinity (MacDonell *et al.*, 1982).

UST030701-295^T differs from the three previously described species of *Winogradskyella* on the basis of: (i) sensitivity to streptomycin and benzylpenicillin, (ii) ability to produce acetoin, (iii) ability to grow at 44 °C and (iv) negative reaction for agar degradation (Table 2). Strain UST030701-295^T can be distinguished from *W. epiphytica*, *W. eximia* and *W. thalassocola* on the basis of seven, 17 and 17 phenotypic properties, respectively (Table 2). Molecular evidence together with phenotypic characteristics suggests that strain UST030701-295^T represents a novel species within the genus *Winogradskyella*.

Description of *Winogradskyella poriferorum* sp. nov.

Winogradskyella poriferorum (por.if.er.or′um. N.L. gen. pl. n. *poriferorum* of the phylum Porifera, referring to the isolation source sponge, of the phylum Porifera).

Cells are Gram-negative, rod-shaped and show rapid gliding motility. After cultivation on marine agar, colonies are yellow, circular, 2–4 mm in diameter, convex with a smooth surface and entire margins. Does not produce flexirubin or diffusible pigment. MK-6 is the only respiratory quinone.

Growth of the type strain is strictly aerobic, and occurs between 12 and 44 °C (but not at 4 or 52 °C) and between pH 6·0 and 10·0. Requires NaCl (1·0–4·0 %) for growth. The G+C content of the DNA is 32·8 mol% and the predominant fatty acids are iso-$C_{15:1}$, iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$ 3-OH, iso-$C_{16:0}$ 3-OH, $C_{16:1}\omega7$ and iso-$C_{17:0}$ 3-OH (together representing 75·4 % of the total). Susceptible to ampicillin (0·5 μg), benzylpenicillin (0·5 μg), chloramphenicol (1·0 μg), streptomycin (10 μg) and tetracycline (0·5 μg). Resistant to kanamycin (tested up to 100 μg). Acetoin is produced, but not indole or H_2S. DNA, gelatin and Tweens 20, 40 and 80 are degraded, but agar, casein, cellulose, chitin or starch are not. Citrate is not utilized. Nitrate is not reduced. Positive for α-chymotrypsin, catalase, cystine arylamidase, leucine arylamidase, valine arylamidase, oxidase, esterase (C_4), esterase lipase (C_8), acid phosphatase, alkaline phosphatase, lipase (C_{14}), naphthol-AS-BI-phosphohydrolase and trypsin activity. Negative for *N*-acetyl-β-glucosaminidase, tryptophan deaminase, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-fucosidase, α-mannosidase and urease activity. Utilizes aesculin as sole carbon source, but none of the other substrates tested in the API 50CH system (see Supplementary Table S2 in IJSEM Online for further details).

The type strain, UST030701-295^T (=NRRL B-41101^T=JCM 12885^T), was isolated from the surface of the sponge *Lissodendoryx isodictyalis* in the Bahamas.

Acknowledgements

This work was supported by RGC grants HKUST6240/04M and CA04/05.Sc01 to P.-Y. Q. and an NSF Biological Oceanography Program grant (OCE-0095724) to J. R. P.

References

Acar, J. F. (1980). The disc susceptibility test. In *Antibiotics in Laboratory and Medicine*, pp. 24–54. Edited by V. Lorian. Baltimore: Williams & Wilkins.

Baumann, P. & Baumann, L. (1981). The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In *The Prokaryotes*, vol. 1, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. Schlegel. Berlin: Springer.

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.

Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

Bowman, J. P., Nichols, C. M. & Gibson, J. A. E. (2003). *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. *Int J Syst Evol Microbiol* **53**, 1343–1355.

Collins, M. D. (1994). Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265–310. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Isnansetyo, A. & Kamei, Y. (2003). *Pseudoalteromonas phenolica* sp. nov., a novel marine bacterium that produces phenolic anti-methicillin-resistant *Staphylococcus aureus* substances. *Int J Syst Evol Microbiol* **53**, 583–588.

Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

MacDonell, M. T., Singleton, F. L. & Hood, M. A. (1982). Diluent composition for use of API 20E in characterizing marine and estuarine bacteria. *Appl Environ Microbiol* **44**, 423–427.

Mesbah, M., Premachandran, U. & Whitman, W. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Nakagawa, Y. & Yamasato, K. (1993). Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K. & 9 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.

Neu, B., Voigt, A., Mitlohner, R. & 7 other authors (2001). Biological cells as templates for hollow microcapsules. *J Microencapsul* **18**, 385–395.

Norris, J. R., Ribbons, D. W. & Varma, A. K. (editors) (1985). *Methods in Microbiology*, vol. 18. London: Academic Press.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characteristics. In *Methods for General and Molecular Biology*, pp. 607–654. Edited by P. Gerhardt, R.G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Steyn, P. L., Segers, P., Vancanneyt, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998). Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. *Int J Syst Bacteriol* **48**, 165–177.