The Journal of Microbiology (2012) Vol. 50, No. 5, pp. 888–892
DOI 10.1007/s12275-012-2447-0

NOTE

Winogradskyella jejuensis sp. nov., a Marine Bacterium Isolated from a Brown Alga *Carpopeltis affinis*

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(Received August 21, 2012 / Accepted September 30, 2012)

A Gram-negative, orange-pigmented, rod-shaped bacterium, designated strain CP32^T was isolated from a brown alga *Carpopeltis affinis* collected on the coast of Jeju Island, Republic of Korea. The isolate grew at 10–37°C (optimum 25°C) and at pH 6.5–9.5 (optimum pH 7.0). The 16S rRNA gene sequence of the isolate showed much similarity with the type strains of recognized species of the genus *Winogradskyella* (94.0–96.6%). The most closely related species were *Winogradskyella echinorum* KMM 6211^T, *Winogradskyella ulvae* KMM 6390^T, *Winogradskyella thalassocola* KMM 3907^T, *Winogradskyella poriferorum* UST030701-295^T, and *Winogradskyella eximia* KMM 3944^T. The major respiratory quinone was menaquinone-6 (MK-6) and the predominant cellular fatty acids were iso-$C_{15:1}$ G (24.8%), iso-$C_{15:0}$ (23.4%), and iso-$C_{17:0}$ 3-OH (11.6 %). The DNA G+C content was 33.3 mol%. The polar lipid profile was composed of phosphatidylethanolamine, two aminolipids, and five unknown lipids. On the basis of phenotypic features, and the result of 16S rRNA gene sequence analysis, strain CP32^T (=KCTC 23835^T =JCM 18454^T) represents a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella jejuensis* sp. nov. is proposed.

Keywords: *Flavobacteriaceae*, *Winogradskyella jejuensis* sp. nov., brown alga

The genus *Winogradskyella*, a member of the family *Flavobacteriaceae* (Bernardet *et al*., 2002; Bernardet, 2011) of the phylum *Bacteroidetes*, was established by Nedashkovskaya *et al*. (2005) with the description of three species, *Winogradskyella thalassocola*, *Winogradskyella epiphytica*, *Winogradskyella eximia*. At the time of writing the manuscript, the *Winogradskyella* genus comprised 9 species : *W. poriferorum* (Lau *et al*., 2005), *W. echinorum* (Nedashkovskaya *et al*., 2009), *W. arenosi* (Romanenko *et al*., 2009), *W. rapida* (Pinhassi *et al*., 2009), *W. pacifica* (Kim and Nedashkovskaya, 2010), *W. exilis* (Ivanova *et al*., 2010), *W. lutea* (Yoon *et al*., 2011), *W. aquimaris* (Lee *et al*., 2012), and *W. ulvae* (Nedashkovskaya *et al*., 2012). Members of the genus *Winogradskyella* have been isolated from seaweeds, marine animals, and the marine environment.

While screening bacterial isolates from brown alga *Carpopeltis affinis* collected from Jeju Island in the Republic of Korea, a novel strain of the genus *Winogradskyella* was isolated, and was taxonomically characterized using a polyphasic approach. The alga was homogenized with sterile seawater, and the homogenate was spread onto marine agar 2216 (MA; Difco, USA) using a dilution-plating technique. After incubation for 7days at 25°C, the purified colonies were preserved in a glycerol solution (20%, w/v) at -70°C and were identified tentatively by partial 16S rRNA gene sequences. Strain CP32^T has been deposited in the Korea Collection for Type Culture (KCTC) and Japan Collection of Microorganisms (JCM).

The 16S rRNA gene was amplified from genomic DNA using bacterial universal 27F and 1522R primers (Weisburg *et al*., 1991). The PCR product was purified using a QIAquick PCR purification kit (QIAGEN, USA) and the PCR product was sequenced by Genotech (Korea). A full sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR, USA). Sequences of related taxa were obtained from the GenBank database and EzTaxon server (Chun *et al*., 2007). Multiple alignments were performed using the CLUSTAL X version 1.83 program (Thompson *et al*., 1997) and gaps were excluded by using the BioEdit program (Hall, 1999). A phylogenetic tree was constructed by using the neighbor-joining (Saitou and Nei, 1987), maximum likelihood (Felsenstein, 1981), and maximum parsimony (Kluge and Farris, 1969) algorithms contained in MEGA version 5.0 (Tamura *et al*., 2011). Bootstrap analysis based on 1,000 resamplings was used to evaluate the reliability of tree topology (Felsenstein, 1985).

Gram staining was performed using the Gram stain kit (BD, USA) according to the manufacturer's instructions. Cell morphology was examined by using light microscopy (Nikon, Japan) and scanning electron microscopy (SEC, Korea). Growth at 4, 10, 15, 20, 25, 30, 37, 40, and 45°C was measured on MA. The pH range for growth was investigated on MA adjusted to pH 4.0–11.0 in increments of 0.5 pH units using 1 M HCl or 1 M NaOH. Salt tolerance was tested by

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The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain CP32^T is JF820844.

incubation for 7 days on Zobell agar medium (Zobell, 1941; 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate and 15 g Bacto agar in 1 L distilled water) supplemented with 1–10% (w/v) NaCl. Catalase activity was observed by bubble production in a 3% solution (v/v) of hydrogen peroxide, and oxidase activity was determined by the oxidation of 1% (w/v) tetramethyl *p*-phenylenediamine (Merck, USA). Hydrolysis of casein, carboxymethylcellulose, starch, as well as Tween 20, 40, 60, and 80 was performed on MA using the methods of Smibert and Krieg (1994). DNase activity was examined using DNase test agar (Difco, USA) with methyl green. Anaerobic growth was investigated using incubation in the AnaeroPack system (Oxoid, UK) for 4 weeks at 25°C on MA.

Other physiological and biochemical tests were carried out using the API 20E, API 20NE, and API ZYM test kits (bioMerieux, UK) and Biolog GN2 (Biolog, USA) according to the manufacturer's instructions. Susceptibility to antibiotics was tested on MA plates using antibiotic discs (BBL, USA) containing the following: ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), erythromycin (15 μg), kanamycin (30 μg), lincomycin (2 μg), neomycin (30 μg), novobiocin (30 μg), penicillin G (10 IU), polymyxin B (300 IU), streptomycin (10 μg), and tetracycline (30 μg).

Fatty acid methyl esters were prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI; version 6.1; TSBA6 library) (Sasser, 1990) and were analyzed by gas chromatography (Agilent, USA). For fatty acid analysis, the CP32^T strain and the type strains of related *Winogradskyella* species were grown on MA at 25°C for 3 days. The major respiratory quinones were analyzed by the Korean Culture Center of Microorganisms (KCCM; Republic of Korea), using reverse-phase HPLC (Tamaoka and Komagata, 1984). Total genomic DNA was extracted and purified according to the method of Marmur (1961). The G+C content of the DNA was determined by the thermal denaturation method (*Tm*) with *Escherichia coli* K-12 used as the control (Marmur and Doty, 1962). Polar lipids were extracted according to the procedure described by Minnikin *et al.* (1984) and were analyzed by two-dimensional thin-layer chromatography (TLC) with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata and Suzuki, 1987).

The 16S rRNA gene sequence of strain CP32^T contained a continuous stretch of 1,441 nucleotides, and was deposited in GenBank under the accession number JF820844. The 16S rRNA sequence similarities using the EzTaxon database (Chun *et al.*, 2007) showed that strain CP32^T was closely related to the genus *Winogradskyella*. Strain CP32^T exhibited 16S rRNA gene sequence similarity values of 96.6, 96.5, 96.0, 95.9, and 95.8% to *Winogradskyella echinorum* KMM 6211^T, *Winogradskyella ulvae* KMM 6390^T, *Winogradskyella thalassocola* KMM 3907^T, *Winogradskyella poriferorum* UST030701-295^T, and *Winogradskyella eximia* KMM 3944^T respectively. Levels of 16S rRNA gene sequence similarity between the isolate and the other *Winogradskyella* species and related taxa were below 96.6%. In a neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, strain CP32^T was positioned at the foot of the cluster comprising

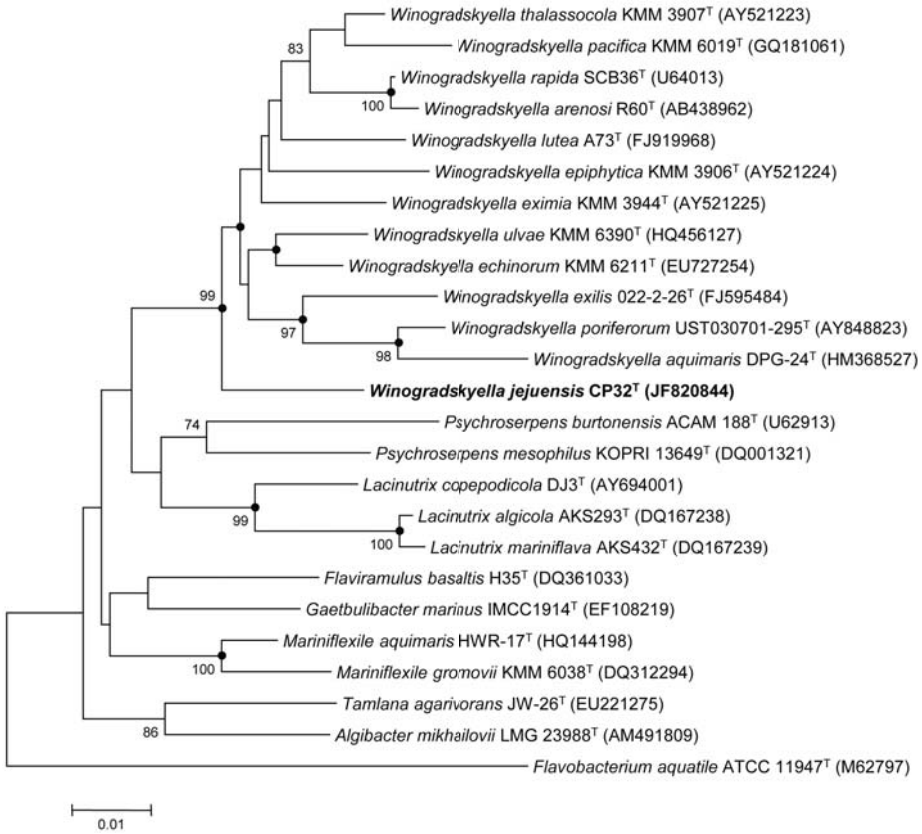

Fig. 1. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences showing the relationships of strain CP32^T with *Winogradskyella* species and other representative members of the family *Flavobacteriaceae*. Black circles indicate that the corresponding branches were recovered in both the maximum parsimony tree and the maximum likelihood tree. GenBank accession nos. are given in parentheses. Bootstrap value (>70%) based on 1,000 replications are shown at branch nodes. *Flavobacterium aquatile* ATCC 11947^T was used as an out-group. Bar, 0.01 changes per nucleotide position.

Table 1. Differential characteristics among strain CP32^T and closely related type strains of genus *Winogradskyella*

Strains: 1, *W. jejuensis* CP32^T; 2, *W. echinorum* KCTC 22026^T; 3, *W. ulvae* KCTC 23626^T; 4. *W. thalasscola* KCTC 12221^T; 5, *W. poriferorum* JCM 12885^T; 6, *W. eximia* KCTC 12219^T. +, Positive; -, negative. All strains were positive for catalase, but negative for H_2S production.

Characteristic	1	2	3	4	5	6
Pigmentation	Orange	Yellow	Yellow	Yellow	Yellow	Yellow
Growth at/ with						
37°C	+	+	+	-	+	-
8% NaCl	-	-	-	+	-	-
Acetoin production	-	-	-	-	+	-
Hydrolysis of:						
Aesculin	+	+	+	+	+	-
Agar	-	-	+	+	-	+
Casein	+	+	+	-	-	+
DNA	-	+	+	-	+	-
Starch	+	-	+	-	-	+
Tween 20	-	-	-	-	+	+
Tween 40	-	-	+	-	+	+
Tween 80	-	-	+	-	+	-
Acid formation from:						
Cellobiose	-	-	-	+	-	-
D-Glucose	-	-	+	+	-	+
Mannitol	-	-	-	-	-	+
Sucrose	-	-	-	-	-	+
Utilization of :						
D-Glucose	-	+	+	+	-	+
D-Mannose	-	+	+	+	-	+
Mannitol	-	-	-	-	-	+
Sucrose	-	-	-	-	-	+
Susceptibility to:						
Ampicillin	+	-	+	+	+	+
Licomycin	+	-	+	+	+	+
Penicillin G	+	-	+	+	+	-
Tetracycline	+	-	+	-	+	-
DNA G+C content (mol%)	33.3	33.6[a]	34.2[a]	34.6[a]	36.1[a]	32.8[a]

[a] DNA G+C content data from Nedashkovskaya *et al.* (2005, 2009, 2012) and Lau *et al.* (2005).

species of the genus *Winogradskyella* with a bootstrap value of 99% (Fig. 1). In the phylogenetic trees reconstructed using both maximum-likelihood and maximum-parsimony algorithms, strain CP32^T fell under the clade encompassed by the genus *Winogradskyella*.

Strain CP32^T was orange-colored with a shiny surface when routinely cultured on MA at 25°C. It was a Gram-negative, aerobic, and rod-shaped bacterium, and was able to grow at temperatures of 10–37°C and in the presence of 1–3% NaCl. Optimum growth occurs at 20–25°C and pH 7.0. The strain is positive for oxidase and catalase activities. The phenotypic and biochemical characteristics that differentiate the strain CP32^T from related *Winogradskyella* species are listed in Table 1.

Profiling of cellular fatty acids revealed that the major fatty acid components of this novel bacterium were iso-$C_{15:1}$ G (24.8%), iso-$C_{15:0}$ (23.4%), iso-$C_{17:0}$ 3-OH (11.2%), iso-$C_{16:0}$ 3-OH (7.7%), Summed feature 3 ($C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c) (6.6%), and iso-$C_{15:0}$ 3-OH (6.1%). This fatty acid profile was similar to those of *Winogradskyella* type strains, although there were differences in the values and presence/absence of some fatty acids (Table 2). The composition of fatty acids of strain CP32^T was similar to those of *W. poriferorum* UST030701-295^T, and the other type strains of different species did not have iso-$C_{16:1}$ G and summed feature 9 ($C_{16:0}$ 10-methyl and/or iso $C_{17:1}$ ω9c).

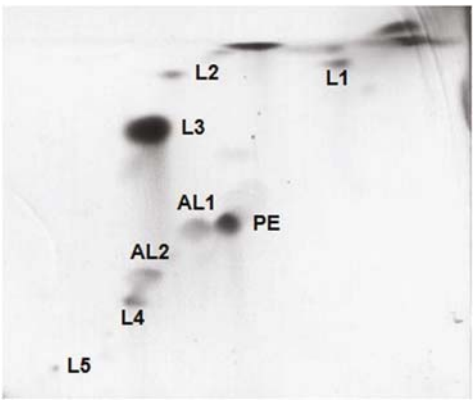

Fig. 2. Two dimensional thin-layer chromatogram sprayed with molybdatophosphoric acid reagent to identify total polar lipids of strain CP32^T. PE, phosphatidylethanolamine; AL 1–2, unknown aminolipids; L 1–4, unknown lipids.

Table 2. Cellular fatty acid profiles of strain CP32^T and the closely related type strains of genus *Winogradskyella*

Strains: 1, *W. jejuensis* CP32^T; 2, *W. echinorum* KCTC 22026^T; 3, *W. ulvae* KCTC 23626^T; 4, *W. thalasscola* KCTC 12221^T; 5, *W. poriferorum* JCM 12885^T; 6, *W. eximia* KCTC 12219^T. All data were obtained during this study from cultures on MA at 25°C for 3 days. Fatty acids that account for less than 0.5% of the total fatty acids in all strains were excluded. tr, trace amount (<1%); -, Not detected.

Fatty acid	1	2	3	4	5	6
iso-$C_{14:0}$	2.1	1.2	tr	1.6	3.2	4.2
$C_{14:0}$	tr	tr	tr	tr	tr	1.2
iso-$C_{15:1}$ G	24.8	21.4	22.6	20.8	28.3	10.2
anteiso-$C_{15:1}$ A	2.0	3.4	2.3	4.9	1.4	1.6
iso-$C_{15:0}$	23.4	27.0	27.1	14.5	17.1	22.5
anteiso-$C_{15:0}$	2.7	6.4	8.4	8.3	1.2	4.4
$C_{15:1}$ ω6c	-	-	-	3.2	-	1.1
iso-$C_{15:0}$ 3-OH	6.1	9.7	8.1	9.1	6.2	4.4
$C_{15:0}$ 2-OH	tr	1.0	1.1	2.1	2.5	tr
$C_{15:0}$ 3-OH	tr	tr	-	1.4	tr	-
$C_{15:0}$	2.1	3.7	5.5	7.1	11.1	4.4
iso-$C_{16:1}$ G	1.9	tr	-	-	2.5	-
iso-$C_{16:1}$ H	-	-	1.4	2.2	-	7.5
iso-$C_{16:0}$	2.2	tr	1.1	tr	3.1	5.5
$C_{16:0}$	1.3	2.4	1.2	1.2	1.1	2.0
iso-$C_{16:0}$ 3-OH	7.7	3.4	4.0	7.8	5.0	14.4
$C_{16:0}$ 3-OH	2.8	tr	-	tr	tr	1.6
iso-$C_{17:0}$ 3-OH	11.2	8.9	6.2	7.1	5.8	4.9
$C_{17:0}$ 2-OH	tr	1.5	1.2	1.4	-	1.1
Summed feature 3[a]	6.6	6.0	6.0	4.5	10.2	6.5
Summed feature 9[a]	-	1.6	2.0	1.5	-	1.1

[a] Summed feature 3 comprised $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c; Summed feature 9 comprised $C_{16:0}$ 10-methyl and/or iso $C_{17:1}$ ω9c.

Strain CP32^T contained menaquinone-6 (MK-6) as the predominant respiratory quinone, which is common to members of the family *Flavobacteriaceae* (Bernardet and Nakagawa, 2006). The G+C content of the genomic DNA was 33.3 mol%, which was within the range described for members of the genus *Winogradskyella* (30.1–36.3 mol%). Strain CP32^T contained phosphatidylethanolamine (PE), unknown aminolipids (AL1-AL2), and unknown lipids (L1-L5) (Fig. 2). The polar lipid profile of strain CP32^T was typical of genus *Winogradskyella* with respect to the presence of phosphatidylethanolamine as the major component. The polar lipid content of strain CP32^T was similar to that of *W. echinorum* KMM 6211^T, *W. ulvae* KMM 6390^T, and *W. thalassocola* KMM 3907^T, but was distinguishable from those of reference strains by the absence of the unknown lipids (L2-L5).

Because strain CP32^T had a relatively low 16S rRNA gene sequence similarity (<97%) to the closely related species of the genus *Winogradskyella*, and represented a distinct phylogenetic position within the genus *Winogradskyella*, DNA-DNA hybridization experiments were not performed (Stackebrandt and Goebel, 1994).

Based on phenotypic distinctness and the result of the 16S rRNA gene sequence analysis, strain CP32^T is considered to represent a novel species of the genus *Winogradskyella*, for which the name *Winogradskyella jejuensis* sp. nov. is proposed.

Description of *Winogradskyella jejuensis* sp. nov.

Winogradskyella jejuensis (je.ju.en'sis. N.L. fem. adj. *jejuensis* referring to Jeju, from where the new species was isolated, Republic of Korea)

Cells are Gram-negative, aerobic, straight or slightly curved rods 0.2–0.3 μm wide and 1.8–4.3 μm long. Colonies on MA are round, 1–1.5 mm in diameter and are orange pigmented with a shiny surface. Grows at 10–37°C, but not at 45°C, and in pH 6–9. Optimum growth occurs at 20–25°C and pH 7.0. Sodium ions are required for growth. Grows on supplemented NaCl to 1–3% (w/v). Aesculin, casein, gelatin, and starch are degraded, but cellulose, Tweens 20, 40, 60, and 80 are not. Citrate is not utilized. Nitrate is reduced to nitrite, and acetoin, indole and H_2S production are negative.

The following compounds are utilized as sole carbon sources: dextrin, I-erythritol, D-galactose, gentiobiose, α-D-glucose-1-phophate, glucuronamide, D-glucuronic acid, glycerol, *m*-inositol, 2-keto-D-gluconic acid, lactulose, α-methyl-D-galactoside, D-psicose, D-xylose, succinamic acid, and uridine. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), cystine-, valine-, and leucine arylamidases, α-chymotrypsin, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase activities are present, but lipase (C14), α–galactosidase and α-glucosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are absent.

Susceptible to ampicillin (1 μg), cephalothin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), licomycin (2 μg), novobiocin (30 μg), penicillin G (10 IU), and tetracycline (30 μg). Resistant to gentamycin (10 μg), kanamycin (30 μg), neomycin (30 μg), polymyxin B (300 IU), and streptomycin (10 μg). The major fatty acids were iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{16:0}$ 3-OH, and iso-$C_{15:0}$ 3-OH. The DNA G+C content is 33.3 mol% and the major respiratory quinone is menaquinone-6. The polar lipids are phosphatidy-

lethanolamine (PE), unknown aminolipids (AL1-AL2), and unknown lipids (L1-L5).

The type strain, CP32^T (= KCTC 23835^T = JCM 18454^T), was isolated from brown alga *Carpopeltis affinis* collected along coast of Jeju Island, Republic of Korea.

References

Bernardet, J.-F. 2011. Family I. *Flavobacteriaceae* Reichenbach 1992. pp. 106–111. Bergey's Manual of Systematic Bacteriology, 2nd ed. *In* Whitman, W. (ed.), The Williams & Wilkins Co., Baltimore, USA.

Bernardet, J.-F. and Nakagawa, Y. 2006. An introduction to the family *Flavobacteriaceae*. pp. 455–480. The Prokaryotes. A Handbook on the Biology of Bacteria, 3rd edn. *In* Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., and Stackebrandt, E. (eds.) Springer, New York, N.Y., USA.

Bernardet, J.-F., Nakagawa, Y., and Holmes, B. 2002. Proposal minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int. J. Syst. Evol. Microbiol.* **52**, 1049–1070.

Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., and Lim, Y.W. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **57**, 2259–2261.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95–98.

Ivanova, E.P., Christen, R., Gorshkova, N.M., Zhukova, N.V., Kurilenko, V.V., Crawford, R.J., and 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. and Nedashkovskaya, O.I. 2010. *Winogradskyella pacifica* sp. nov., a marine bacterium of the family *Flavobacteriaceae*. *Int. J. Syst. Evol. Microbiol.* **60**, 1948–1951.

Kluge, A.G. and Farris, F.S. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**, 1–32.

Komagata, K. and Suzuki, K. 1987. Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19**, 161–207.

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., and 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.

Lee, S.Y., Park, S., Oh, T.K., and Yoon, J.H. 2012. *Winogradskyella aquimaris* sp. nav., isolated from seawater. *Int. J. Syst. Evol. Microbiol.* **62**, 1814–1818.

Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* **3**, 208–218.

Marmur, J. and Doty, P. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**, 109–118.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Nedashkovskaya, O.I., Kim, S.B., Han, S.K., Snauwaert, C., Vancanneyt, M., Swings, J., Kim, K.-O., Lysenko, A.M., Rohde, M., Frolova, G.M., and *et al.* 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., Kukhlevskiy, A.D., and Zhukova, N.V. 2012. *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*. *Int. J. Syst. Evol. Microbiol.* **62**, 1450–1456.

Nedashkovskaya, O.I., Vancanneyt, M., Kim, S.B., and 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, A., and 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., and 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. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. : MIDI Inc., Newark, DE, USA.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization. Methods for General and Molecular Bacteriology. pp. 607–654. *In* Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.). American Society for Microbiology, Washington, D.C., USA.

Stackebrandt, E. and Goebel, B.M. 1994. A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Evol. Microbiol.* **44**, 846–849.

Tamaoka, J. and Komagata, K. 1984. Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**, 125–128.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703.

Yoon, B.J., Byun, H.D., Kim, J.Y., Lee, D.H., Kahng, H.Y., and Oh, D.C. 2011. *Winogradskyella lutea* sp. nov., isolated from seawater, and emended description of the genus *Winogradskyella*. *Int. J. Syst. Evol. Microbiol.* **61**, 1539–1543.

ZoBell, C.E. 1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J. Mar. Res.* **4,** 42–75.