Hyunsoonleella jejuensis gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from seawater

Byoung-Jun Yoon,^{1†} Dong-Heon Lee,^{1,2†} Bong-Jo Kang,³
Hyung-Yeel Kahng,² You-Sung Oh,¹ Jung-Hoon Sohn,⁴
Eui-Sung Choi⁴ and Duck-Chul Oh¹

Correspondence

Duck-Chul Oh

duckoh@jejunu.ac.kr

¹Department of Biology, College of Natural Science, Jeju National University, Jeju 690-756, Republic of Korea

²Department of Environmental Education, Sunchon National University, Sunchon 540-742, Republic of Korea

³Jeju Province Fisheries Resources Research Institute, Jeju 699-915, Republic of Korea

⁴Laboratory System Microbiology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-333, Republic of Korea

A novel marine, Gram-staining-negative, yellow-pigmented, rod-shaped bacterial strain, designated CNU004^T, was isolated from a seawater sample collected on the coastline of Jeju Island, South Korea. The strain was strictly aerobic, non-flagellated, non-gliding and oxidase- and catalase-positive. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CNU004^T belongs to a distinct lineage in the family *Flavobacteriaceae*. Strain CNU004^T exhibited levels of 16S rRNA gene sequence similarity of 93.8–93.9% to its nearest phylogenetic neighbours, members of the genera *Gaetbulibacter*, *Yeosuana* and *Algibacter*. The new isolate required sea salts or artificial seawater for growth. The optimum ranges of temperature and pH for growth were 30–35 °C and pH 7.0–8.0. The DNA G + C content of strain CNU004^T was 37.7 mol%. The major fatty acids were iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and iso-C_{15:0} 3-OH. Menaquinone-6 was the major respiratory quinone. Zeaxanthin was the major carotenoid pigment produced, and flexirubin-type pigments were not produced. Strain CNU004^T was able to degrade starch and agar. Based on its phenotypic and genotypic characteristics and on the phylogenetic evidence presented, strain CNU004^T is considered to represent a novel species of a new genus in the family *Flavobacteriaceae*, for which the name *Hyunsoonleella jejuensis* gen. nov., sp. nov. is proposed. The type strain of *Hyunsoonleella jejuensis* sp. nov. is CNU004^T (=KCTC 22242^T =DSM 21035^T).

The family *Flavobacteriaceae* (Reichenbach, 1989; Bernardet *et al.*, 2002; Bernardet & Nakagawa, 2006) is one of the major phylogenetic lineages within the phylum *Bacteroidetes* (Garrrity & Holt, 2001). At the time of writing, the family comprises more than 50 recognized genera. Many members of this family were isolated from various marine ecosystems and Antarctic habitats (Bowman *et al.*, 1997, 1998; Bowman, 2000; Bowman & Nichols, 2002, 2005; Cho & Giovannoni, 2004; Nedashkovskaya *et al.*, 2004; Nichols *et al.*, 2005; Pinhassi *et al.*, 2006). Members of most genera in the family *Flavobacteriaceae* produce light- to bright-yellow pigments. These pigments may

belong to the carotenoid and/or the flexirubin types depending on the genus (Bernardet *et al.*, 2002).

Various bacteria are known to produce zeaxanthin, including *Synechocystis* sp. strain PCC 6803 (Lagarde *et al.*, 2000), *Erwinia herbicola* (Hundle *et al.*, 1993), *Paracoccus zeaxanthinifaciens* (Berry *et al.*, 2003), *Sphingobacterium multivorum* (Alcantara & Sanchez, 1999) and *Zeaxanthinibacter enoshimensis* (Asker *et al.*, 2007). In the present study, we report the isolation, identification and characterization of a novel zeaxanthin-producing marine bacterium isolated from seawater.

Strain CNU004^T was isolated from a seawater sample collected along the coastline of Jeju Island, Republic of Korea. Serially diluted seawater samples were spread on marine agar 2216 (MA; Difco) and incubated at 30 °C for

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CNU004^T is EF217419.

7 days. Strain CNU004^T formed small, irregular, yellow-pigmented colonies on MA plates after 4 days incubation. The strain was preserved at -80°C in marine broth 2216 (MB; Difco) supplemented with 20 % glycerol (v/v).

Genomic DNA was extracted and purified by using the Wizard genomic DNA purification kit (Promega) according to the manufacturer's instructions, and the nearly complete 16S rRNA gene (1517 bp) was amplified by using bacterial universal primers (Johnson, 1994). The 16S rRNA gene fragment was sequenced directly by using a BigDye Terminator version 3.1 cycle sequencing kit and an ABI 3730xl automated DNA sequencer (Applied Biosystems).

The 16S rRNA gene sequence of strain CNU004^T was compared with those available in GenBank via the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine an approximate phylogenetic affiliation, and the gene sequence was then aligned with those of closely related species by using the CLUSTAL W software program (Thompson *et al.*, 1994). Sequence similarity values were computed by using Similarity Matrix version 1.1 (Ribosomal Database Project II, <http://rdp.cme.msu.edu/index.jsp>; Cole *et al.*, 2003) and the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Phylogenetic trees were constructed by using the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms available in the PHYLIP software, version 3.6 (Felsenstein, 2002). Evolutionary distance matrices were calculated according to the algorithm of Kimura's two-parameter model for the neighbour-joining method. Bootstrap analysis (1000 replications) was performed, by using the neighbour-joining method in the PHYLIP package, to evaluate the stability of the phylogenetic tree.

The neighbour-joining tree (Fig. 1) showed that strain CNU004^T forms a branch related to but distinct from the genera *Formosa*, *Bizionia*, *Algibacter*, *Psychroserpens*, *Winogradskyella*, *Tamlana*, *Marinifexile*, *Gaetbulibacter*, *Yeosuana* and *Gelidibacter*. The distinct phylogenetic position of strain CNU004^T was confirmed in the maximum-likelihood and maximum-parsimony trees (not shown). Levels of pairwise 16S rRNA gene sequence similarity between strain CNU004^T and the type strains of members of related genera were $\leq 93.9\%$. Strain CNU004^T exhibited 16S rRNA gene sequence similarities of 93.8–93.9 % to its nearest phylogenetic neighbours, members of the genera *Gaetbulibacter*, *Yeosuana* and *Algibacter*.

Cell morphology and the presence of flagella were investigated by light microscopy (FDX-35; Nikon) and transmission electron microscopy (JEM100; JEOL) by using cells grown on MA at 30°C for 4 days. Gliding motility was assessed on $0.1 \times \text{MA}$ (containing 10 % MB and 1 % agar) plates (Bowman, 2000) by using the hanging-drop method (Bernardet *et al.*, 2002). The Gram reaction was examined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at 5, 10, 15, 20, 25, 30, 35 and 40°C was measured on MA, while growth at pH 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5 and 11 and tolerance of 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 7 % (w/v) NaCl were measured in MB. Tolerance of sea salts and artificial seawater was determined on trypticase soy agar (TSA; Difco) supplemented with 0–12 % (w/v; 1 % intervals) sea salts (Sigma) and 10–200 % (v/v) artificial seawater (Yoon *et al.*, 2002). Growth with NaCl as the sole salt was studied on TSA supplemented with 0–12 % (w/v) NaCl. Anaerobic growth was assessed on MA incubated in a GasPak anaerobic system (BBL) for 7 days at 30°C . The presence of catalase activity was revealed by the production of oxygen bubbles by the cells when mixed with

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain CNU004^T and related taxa. Bootstrap percentages (based on 1000 replicates) $>50\%$ are shown at branching points. The sequence of *Brumimicrobium glaciale* ACAM 644^T (GenBank accession no. AF521195) was used as an outgroup (not shown). Bar, 0.02 changes per nucleotide position.

3 % (v/v) H_2O_2 on a glass slide. Oxidase activity was determined based on oxidation of 1 % (w/v) N,N,N',N' -tetramethyl-*p*-phenylenediamine solution. DNase activity was examined by using DNase test agar with methyl green (Difco) supplemented with 2 % (w/v) sea salts (Sigma). Hydrolysis of casein, starch and Tween 80 was tested on MA by using the substrate concentrations given by Cowan & Steel (1965). Hydrolysis of agar and CM-cellulose was investigated as described by Cowan & Steel (1993). Hydrolysis of aesculin, gelatin and urea and reduction of nitrate were investigated as described by Lányi (1987) by using media supplemented with 2 % (w/v) sea salts. H_2S production was tested as described by Bruns *et al.* (2001). Other biochemical tests were performed by using API 20NE and API 20E kits (bioMérieux). Enzyme activities were tested by using an API ZYM kit (bioMérieux) following the manufacturer's instructions. API strips were inoculated with a heavy bacterial suspension in artificial seawater (Yoon *et al.*, 2002) and were incubated at 30 °C for 96 h. The biochemical, physiological and morphological characteristics of strain CNU004^T are given in the genus and species descriptions below and in Table 1.

The presence of flexirubin-type pigments was investigated as described by Bernardet *et al.* (2002). Carotenoids in acetone extracts were detected spectroscopically by using a Shimadzu UV-visible spectrophotometer (model UV-1650PC). Carotenoid composition was characterized by

using HPLC-MS (LCMS-2010EV; Shimadzu) with a Shim-Pack FC-ODS (150 × 4.6 mm, 5- μm particle size; column temperature 35 °C) and acetonitrile/methanol/tetrahydrofuran (5.8:3.5:0.7) as the mobile phase at a flow rate of 0.8 ml min⁻¹. Zeaxanthin was identified based on its retention time, absorption spectrum and molecular mass. Commercial zeaxanthin (DHI; Water & Environment) was used as a standard. Flexirubin-type pigments were absent. The yellow pigments of strain CNU004^T were identified as carotenoids, among which zeaxanthin was predominant.

Cellular fatty acid methyl esters were prepared from cultures grown on MA at 30 °C for 4 days and were analysed by the Korean Culture Center of Microorganisms (KCCM; Seoul, Republic of Korea) by using the MIDI Microbial Identification System. The three closest relatives of strain CNU004^T, *Gaetbulibacter saemankumensis* KCTC 12379^T, *Yeosuana aromativorans* JCM 12862^T and *Algibacter mikhailovii* LMG 23998^T, were grown under the same conditions and were included in the fatty acid analysis. The DNA G + C content and respiratory quinones were also analysed by the KCCM, by using reversed-phased HPLC (Komagata & Suzuki, 1987).

The dominant fatty acids of strain CNU004^T were iso- $\text{C}_{15:0}$ (18.4 % of the total), iso- $\text{C}_{15:1}$ G (17.7 %), iso- $\text{C}_{17:0}$ 3-OH (16.6 %) and iso- $\text{C}_{15:0}$ 3-OH (11.6 %). The detailed fatty acid profile of strain CNU004^T is compared with

Table 1. Differential phenotypic characteristics between strain CNU004^T and related taxa

Strains: 1, CNU004^T; 2, *Yeosuana aromativorans* JCM 12862^T (data from Kwon *et al.*, 2006); 3, *Algibacter mikhailovii* LMG 23998^T (Nedashkovskaya *et al.*, 2007); 4, *Gaetbulibacter saemankumensis* KCTC 12379^T (Jung *et al.*, 2005). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4
Pigmentation	Yellow	Yellowish brown	Pale yellow	Yellow
Gliding motility	–	–	+	+
Ranges (optima) for growth				
NaCl (% w/v)	0.5–4.5 (2–3)	0.5–4.0 (2)	1–6 (2–3)	0–7 (2–3)
Temperature (°C)	15–35 (30–35)	23–39 (33–36)	4–37 (23–25)	13–40 (25–30)
pH	5.5–9.5 (7.0–8.0)	5–8 (7)	ND	≥5.5 (7.0–8.0)
Requirement for:				
Oxygen	+	+	+	–
Sea salts	+	+	ND	–
Production of:				
Oxidase	+	–	+	+
Nitrate reductase	–	–	+	+
Acid from carbohydrates	–	–	–	+
Hydrolysis of:				
Aesculin	+	ND	+	–
Agar	–	–	+	ND
DNA	–	ND	+	ND
Gelatin	+	+	+	–
Starch	–	–	–	+
DNA G + C content (mol%)	37.7	51.4	35.1	34.7–34.9*
Major menaquinone(s)	MK-6	MK-5, MK-6	ND	MK-6

*Range of values for three strains, including the type strain.

those of closely related members of the family *Flavobacteriaceae* in Table 2. The DNA G+C content of strain CNU004^T was 37.7 mol% and its major respiratory quinone was menaquinone-6.

Based on the results of 16S rRNA gene sequence analysis and on phenotypic characteristics, we conclude that strain CNU004^T represents a novel species of a new genus in the family *Flavobacteriaceae*, for which the name *Hyunsoonleella jejuensis* gen. nov., sp. nov. is proposed.

Description of *Hyunsoonleella* gen. nov.

Hyunsoonleella (Hy.un.soon.le.el'la. L. dim. ending -ella; N.L. fem. n. *Hyunsoonleella* named after Hyun-Soon Lee, a Korean microbiologist who devoted her life to the study of photosynthetic bacteria).

Cells are strictly aerobic, Gram staining-negative, yellow-pigmented, non-flagellated and rod-shaped. Gliding motility

is not observed. Oxidase- and catalase-positive. Cells produce non-diffusible carotenoid pigments but flexirubin-type pigments are absent. The major respiratory quinone is menaquinone-6. The cellular fatty acid composition is dominated by branched saturated, branched monounsaturated and hydroxy components. As determined by 16S rRNA gene sequence analysis, the genus *Hyunsoonleella* is a member of the family *Flavobacteriaceae*. The type species is *Hyunsoonleella jejuensis*.

Description of *Hyunsoonleella jejuensis* sp. nov.

Hyunsoonleella jejuensis (je.ju.en'sis. N.L. fem. adj. *jejuensis* pertaining to Jeju Island in the Republic of Korea, from where the type strain was isolated).

Displays the following characteristics in addition to those given for the genus. Cells are 0.3–0.6 µm wide and 1.8–2.2 µm long. After 4 days on MA, colonies are 1.0–1.5 mm in diameter, convex and circular with slightly irregular edges. Cells contain non-diffusible yellow carotenoid pigments with zeaxanthin as the major component. Growth occurs at 15.0–35.0 °C (optimum, 30.0–35.0 °C) and at pH 5.5–9.5 (optimum, pH 7.0–8.0). Na⁺ alone does not support growth. Requires sea salts or artificial seawater for growth. Grows on TSA supplemented with 3–7 % (w/v) sea salts or 20–180 % (v/v) artificial seawater. Grows on MB supplemented with 0–1.5 % (w/v) NaCl. Aesculin, Tween 80, gelatin and CM-cellulose are hydrolysed, but agar, DNA, casein, starch and urea are not. Acetoin is produced, but indole and H₂S are not. Nitrate is not reduced. Tryptophan deaminase, lysine decarboxylase and ornithine decarboxylase activities are absent. Acid is not produced from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin or arabinose. Turanose, mannose, mannitol, gluconate, adipic acid and malate are assimilated, but arabinose, glucose, maltose, *N*-acetylglucosamine, capric acid, trisodium citrate and phenylacetic acid are not. In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, α-galactosidase, β-galactosidase and *N*-acetyl-β-glucosaminidase, and weakly positive for naphthol-AS-BI-phosphohydrolase; negative for lipase (C14), α-chymotrypsin, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. The major fatty acids are iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and iso-C_{15:0} 3-OH. The DNA G+C content of the type strain is 37.7 mol%.

The type strain, CNU004^T (=KCTC 22242^T =DSM 21035^T), was isolated from seawater off Jeju Island, Republic of Korea.

Table 2. Cellular fatty acid content of strain CNU004^T and closely related taxa

Strains: 1, CNU004^T; 2, *Yeosuana aromativorans* JCM 12862^T; 3, *Algibacter mikhailovii* LMG 23998^T; 4, *Gaetbulibacter saemankuensis* KCTC 12379^T. Values are percentages of the total fatty acids. Fatty acids amounting to <1 % of the total in all strains listed are not shown. All data are from the present study.

Fatty acid	1	2	3	4
Saturated				
C _{15:0}	2.0	4.3	—	3.4
C _{16:0}	1.2	1.2	1.7	2.3
Branched saturated				
iso-C _{13:0}	—	—	—	1.8
iso-C _{15:0}	18.4	20.1	13.2	23.1
anteiso-C _{15:0}	4.6	13.7	11.0	6.2
Monounsaturated				
C _{15:1} ω6c	—	—	6.9	—
C _{16:1} ω5c	—	8.1	—	—
C _{17:1} ω6c	—	—	4.3	1.0
Branched monounsaturated				
anteiso-C _{15:1}	3.1	—	—	—
iso-C _{15:1} G	17.7	5.1	10.6	5.9
iso-C _{15:1} ω10c	—	6.8	—	—
iso-C _{17:1} ω9c	—	—	—	1.7
Hydroxy				
C _{15:0} 2-OH	1.4	1.0	1.9	1.0
C _{17:0} 2-OH	3.0	1.2	1.2	2.1
C _{15:0} 3-OH	—	—	1.9	—
C _{16:0} 3-OH	—	—	1.3	—
iso-C _{15:0} 3-OH	11.6	2.2	4.9	5.3
iso-C _{16:0} 3-OH	6.1	1.0	2.0	1.4
iso-C _{17:0} 3-OH	16.6	5.9	6.9	17.9
Summed feature 3*	5.0	6.1	7.0	7.2

*Summed features are groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 3 comprised iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

References

- Alcantara, S. & Sanchez, S. (1999). Influence of carbon and nitrogen sources on *Flavobacterium* growth and zeaxanthin biosynthesis. *J Ind Microbiol Biotechnol* **23**, 697–700.

- Asker, D., Beppu, T. & Ueda, K. (2007). *Zeaxanthinibacter enoshimensis* gen. nov., sp. nov., a novel zeaxanthin-producing marine bacterium of the family *Flavobacteriaceae*, isolated from seawater off Enoshima Island, Japan. *Int J Syst Evol Microbiol* **57**, 837–843.
- Bernardet, J.-F. & Nakagawa, Y. (2006). An introduction to the family *Flavobacteriaceae*. In *The Prokaryotes. A Handbook on the Biology of Bacteria*, 3rd edn, vol. 7, pp. 455–480. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: 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.
- Berry, A., Janssens, D., Hübner, M., Jore, J. P. M., Hoste, B., Cleenwerck, I., Vancanneyt, M., Bretzel, W., Mayer, A. F. & other authors (2003). *Paracoccus zeaxanthinifaciens* sp. nov., a zeaxanthin-producing bacterium. *Int J Syst Evol Microbiol* **53**, 231–238.
- 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, D. S. (2002). *Aequorivita* gen. nov., a member of the family *Flavobacteriaceae* isolated from terrestrial and marine Antarctic habitats. *Int J Syst Evol Microbiol* **52**, 1533–1541.
- Bowman, J. P. & Nichols, D. S. (2005). Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwiliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*. *Int J Syst Evol Microbiol* **55**, 1471–1486.
- Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.
- Bowman, J. P., McCammon, S. A., Lewis, T., Skerratt, J. H., Brown, J. L., Nichols, D. S. & McMeekin, T. A. (1998). *Psychroflexus torquus* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. *Microbiology* **144**, 1601–1609.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda rustringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Cho, J.-C. & Giovannoni, S. J. (2004). *Robiginitalea biformata* gen. nov., sp. nov., a novel marine bacterium in the family *Flavobacteriaceae* with a higher G+C content. *Int J Syst Evol Microbiol* **54**, 1101–1106.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & 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.
- Cole, J. R., Chai, B., Marsh, T. L., Farris, R. J., Wang, Q., Kulam, S. A., Chandra, S., McGarrell, D. M., Schmidt, T. M. & other authors (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Cowan, S. T. & Steel, K. J. (1993). *Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.
- Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Garrity, G. M. & Holt, J. G. (2001). The road map to the *Manual*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 119–166. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Hundle, B. S., O'Brien, D. A., Beyer, P., Kleinig, H. & Hearst, J. E. (1993). *In vitro* expression and activity of lycopene cyclase and β -carotene hydroxylase from *Erwinia herbicola*. *FEBS Lett* **315**, 329–334.
- Johnson, J. L. (1994). Similarity analysis of rRNAs. In *Methods for General and Molecular Bacteriology*, pp. 683–700. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Jung, S.-Y., Kang, S.-J., Lee, M.-H., Lee, S.-Y., Oh, T.-K. & Yoon, J.-H. (2005). *Gaetbulibacter saemankumensis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a tidal flat sediment in Korea. *Int J Syst Evol Microbiol* **55**, 1845–1849.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kwon, K. K., Lee, H.-S., Jung, H.-B., Kang, J.-H. & Kim, S.-J. (2006). *Yeosuana aromativorans* gen. nov., sp. nov., a mesophilic marine bacterium belonging to the family *Flavobacteriaceae*, isolated from estuarine sediment of the South Sea, Korea. *Int J Syst Evol Microbiol* **56**, 727–732.
- Lagarde, D., Beuf, L. & Vermaas, W. (2000). Increased production of zeaxanthin and other pigments by application of genetic engineering techniques to *Synechocystis* sp. strain PCC 6803. *Appl Environ Microbiol* **66**, 64–72.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, A. M., Rohde, M., Rhee, M.-S., Frolova, G. M., Falsen, E., Mikhailov, V. V. & Bae, K. S. (2004). *Maribacter* gen. nov., a new member of the family *Flavobacteriaceae*, isolated from marine habitats, containing the species *Maribacter sedimenticola* sp. nov., *Maribacter aquivivus* sp. nov., *Maribacter orientalis* sp. nov. and *Maribacter ulvicola* sp. nov. *Int J Syst Evol Microbiol* **54**, 1017–1023.
- Nedashkovskaya, O. I., Vancanneyt, M., Kim, S. B., Hoste, B. & Bae, K. S. (2007). *Algibacter mikhailovii* sp. nov., a novel marine bacterium of the family *Flavobacteriaceae*, and emended description of the genus *Algibacter*. *Int J Syst Evol Microbiol* **57**, 2147–2150.
- Nichols, C. M., Bowman, J. P. & Guezennec, J. (2005). *Olleya marilimosa* gen. nov., sp. nov., an exopolysaccharide-producing marine bacterium from the family *Flavobacteriaceae*, isolated from the Southern Ocean. *Int J Syst Evol Microbiol* **55**, 1557–1561.
- Pinhassi, J., Bowman, J. P., Nedashkovskaya, O. I., Lekunberri, I., Gomez-Consarnau, L. & Pedrós-Alí, C. (2006). *Leeuwenhoekiella blandensis* sp. nov., a genome-sequenced marine member of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **56**, 1489–1493.
- Reichenbach, H. (1989). The order *Cytophagales* Leadbetter 1974, 99^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2011–2073. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. C. Holt. Baltimore: Williams & Wilkins.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Yoon, J. H., Lee, K. C., Kho, Y. H., Kang, K. H., Kim, C. J. & Park, Y. H. (2002). *Halomonas alimentaria* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **52**, 123–130.