Cellulophaga pacifica sp. nov.

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Three marine, heterotrophic, aerobic, agarolytic, pigmented and gliding bacteria were isolated in June 2000 from a sea water sample that was collected in the Gulf of Peter the Great, Sea of Japan, and analysed in a polyphasic taxonomic study. 16S rDNA sequence analysis indicated that strains KMM 3664^T, KMM 3669 and KMM 3915 were members of the family *Flavobacteriaceae*. Based on phenotypic, chemotaxonomic, genotypic and phylogenetic data, the isolates were classified in the genus *Cellulophaga* as members of a novel species, *Cellulophaga pacifica* sp. nov. The type strain is KMM 3664^T (=JCM 11735^T=LMG 21938^T).

The genus *Cellulophaga* was proposed by Johansen *et al.* (1999) as a separate branch in the family *Flavobacteriaceae*, to comprise three species: *Cellulophaga baltica* and *Cellulophaga fucicola*, two agarolytic, yellow–orange-pigmented bacteria associated with the brown alga *Fucus serratus*, and *Cellulophaga lytica*, a common representative of coastal microbial communities that was formerly misclassified as [*Cytophaga*] *lytica* (Lewin, 1969; Reichenbach, 1989). The latter species was assigned as the type species of the genus. A fourth member of the genus, *Cellulophaga algicola*, which originated from the surface of algal species from Antarctic marine coasts and sea ice, was described relatively recently (Bowman, 2000). This author also reclassified [*Cytophaga*] *uliginosa* (Reichenbach, 1989), formerly *Flavobacterium uliginosum* (ZoBell & Upham, 1944), in the genus *Cellulophaga* as *Cellulophaga uliginosa*. Recently, *C. uliginosa* has been transferred to a novel genus, *Zobellia*, based on DNA G+C content, maximum growth temperature, presence of flexirubin and phylogenetic position (Barbeyron *et al.*, 2001).

Three gliding, agarolytic, strictly aerobic, Gram-negative and yellow-pigmented bacterial strains were isolated from a sea water sample that was collected in the Sea of Japan, Pacific Ocean. Phenotypic, chemotaxonomic and genotypic characteristics assigned these bacteria to the family *Flavobacteriaceae*. Phylogenetic and phenotypic data indicated that the unknown organisms comprised a distinct species within the genus *Cellulophaga*. The name *Cellulophaga pacifica* sp. nov. is proposed for these sea water isolates; the type strain is KMM 3664^T (=JCM 11735^T=LMG 21938^T).

Strains KMM 3664^T, KMM 3669 and KMM 3915 were isolated from a sea water sample that was collected during June 2000 in Amursky Bay, Gulf of Peter the Great, Sea of Japan, Pacific Ocean, from a depth of 5 m (salinity, 33 ‰; temperature, 15 °C). After primary isolation and purification, strains were cultivated at 28 °C on marine agar 2216 (Difco) and stored at −80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol. The bacteria isolated in this study and reference strains are shown in Table 1.

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Abbreviations: ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Tasmania, Australia; ATCC, American Type Culture Collection, Manassas, VA, USA; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako, Japan; KMM, Collection of Marine Microorganisms of the Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia; LMG, BCCM/LMG Bacteria Collection, Laboratorium Microbiologie, Universiteit Gent, Belgium; NN, Enzyme Research, Novo Nordisk A/S, Bagsvaerd, Denmark.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of *Cellulophaga pacifica* KMM 3664^T, KMM 3669 and KMM 3915 are AB100840, AB100841 and AB100842, respectively.

Table 1. Bacterial strains used in this study

Taxon and strain	Isolation source and location
Cellulophaga pacifica:	
KMM 3664^T	Sea water, Sea of Japan, Pacific Ocean
KMM 3669	Sea water, Sea of Japan, Pacific Ocean
KMM 3915	Sea water, Sea of Japan, Pacific Ocean
Cellulophaga lytica ATCC 23178^T	Beach mud, Costa Rica, Pacific Ocean
Cellulophaga fucicola NN015860^T	Brown alga *Fucus serratus*, North Sea, Atlantic Ocean
Cellulophaga baltica NN015840^T	Brown alga *Fucus serratus*, North Sea, Atlantic Ocean
Cellulophaga algicola ACAM 630^T	Alga from marine coast and sea ice, Antarctica

To detect the precise taxonomic position of the strains studied, the almost-complete 16S rDNA sequences of strains KMM 3664^T, KMM 3669 and KMM 3915 were determined by PCR amplification and direct sequencing (Hiraishi, 1992), using conditions and reagents that were described previously (Suzuki *et al.*, 2001). The determined sequences were aligned to an alignment based on a secondary structure model, maintained by the SSU rRNA database (Van de Peer *et al.*, 2000), by using the profile alignment program of the CLUSTAL W software (Thompson *et al.*, 1994). Evolutionary distances were then computed with the DNADIST program in the PHYLIP 3.572 package (Felsenstein, 1995) with the Kimura two-parameter correction (Kimura, 1980); a phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). To evaluate the phylogenetic tree, bootstrap analysis with 1000 sample replications was performed with the SEQBOOT and CONSENSE programs in the PHYLIP 3.572 package.

Comparative analysis of the sequences obtained in this study with published sequences of representatives of the phylum *Cytophaga–Flavobacterium–Bacteroides* that have been described to date revealed that the sea water isolates were members of the family *Flavobacteriaceae* and formed a distinct lineage within the genus *Cellulophaga* (Fig. 1). 16S rDNA sequence similarity values of strains KMM 3664^T, KMM 3669 and KMM 3915 to the type strains of *C. lytica* and *C. fucicola* were too low (91·9–92·1 and 93·0–93·1 %, respectively) to consider the new isolates as members of these species. However, the new isolates shared a level of 16S rDNA sequence similarity that may indicate possible species-level relatedness to the type strains of *C. algicola* and *C. baltica* (97·1 and 97·5 %, respectively).

For determination of DNA G+C content and DNA–DNA binding values, DNA was prepared from cells that had been cultivated on marine agar (Difco) for 24–48 h at 25 °C. The DNA G+C content was determined by using two approaches: (i) the thermal denaturation method of Marmur & Doty (1962) for strains KMM 3664^T, KMM 3669 and KMM 3915, using the DNA extraction protocol of Marmur (1961); and (ii) the HPLC method of Mesbah *et al.* (1989) for *C. baltica* NN015840^T, KMM 3664^T and

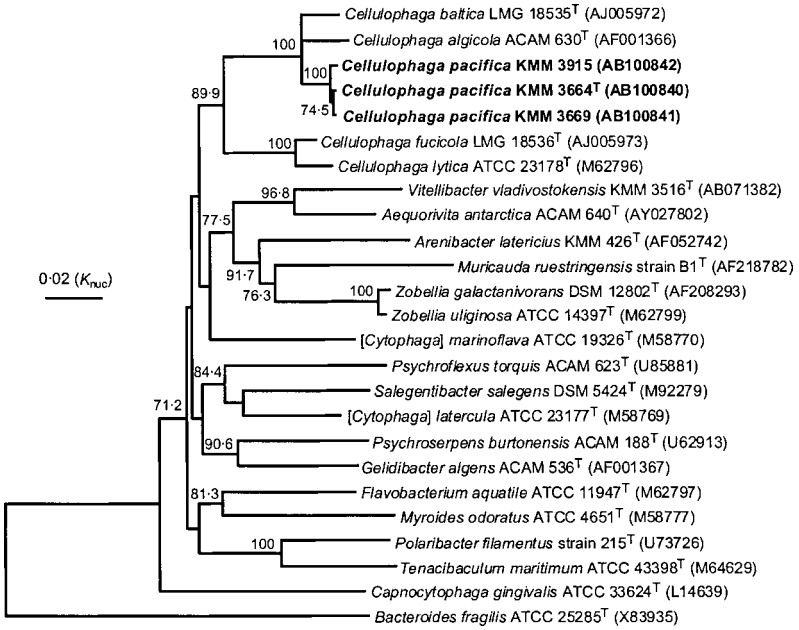

Fig. 1. Phylogenetic position of strains KMM 3664^T, KMM 3669 and KMM 3915 among species of the family *Flavobacteriaceae* on the basis of 16S rDNA sequence comparison. The phylogenetic tree was generated by the neighbour-joining method (Saitou & Nei, 1987). The 16S rDNA sequence of *Bacteroides fragilis* (GenBank accession no. X83935) was used as the outgroup. Numbers next to nodes indicate percentage bootstrap values from 1000 replicates (only values of 70 % or higher are cited). Bar, 0·02 genetic distance (K_{nuc}).

KMM 3669, following the DNA extraction protocol of Pitcher *et al.* (1989) as modified by Leisner *et al.* (2002) and, for *C. algicola* ACAM 630^T, by using the DNA extraction protocol of Wilson (1987) as modified by Cleenwerck *et al.* (2002). These high-molecular-mass DNA extracts were used to perform DNA–DNA hybridizations, using the microplate method and fluorescence measurements for calculation of binding values as described by Ezaki *et al.* (1989). Hybridizations were performed at 35 °C in hybridization mixture (2 × SSC, 5 × Denhardt's solution, 2·5 % dextran sulphate, 50 % formamide, 100 μg denaturated salmon sperm DNA ml^{-1}, 1250 ng biotinylated probe DNA ml^{-1}).

DNA–DNA hybridization experiments indicated that strains KMM 3664^T, KMM 3669 and KMM 3915 shared only 11–12 % DNA–DNA homology with *C. baltica* NN015840^T and *C. algicola* ACAM 630^T. Furthermore, the three new isolates represented one genomic group, with DNA–DNA binding values of 72–90 % between them. DNA–DNA relatedness between *C. algicola* and *C. baltica* was 32 %; this confirms the results of Bowman (2000). DNA G+C contents were 33·2, 33·0 and 34·3 mol% for strains KMM 3664^T, KMM 3669 and KMM 3915, respectively, when determined by the thermal denaturation method. Slightly lower values of 31·6, 32·0, 33·4 and 34·6 mol% for KMM 3664^T, KMM 3669, *C. algicola* ACAM 630^T and *C. baltica* NN015840^T, respectively, were observed when determined by HPLC.

For determination of whole-cell fatty acid composition, strains were cultivated at 21 °C for 48 h on marine agar 2216 (Difco). Analysis of fatty acid methyl esters was performed by GLC [30 m × 0·25 mm Supelcowax 10 column, 205 °C] as described by Svetashev *et al.* (1995).

The predominant cellular fatty acids of strains KMM 3664^T, KMM 3669 and KMM 3915 were branched-chain saturated and unsaturated fatty acids and straight-chain saturated and monounsaturated fatty acids, namely i-$C_{15:0}$ (9·1, 6·1 and 9·6 %, respectively), i-$C_{15:1}$ (20·9, 13·1 and 19·6 %, respectively), $C_{15:0}$ (16·6, 12·0 and 16·9 %, respectively), $C_{16:1}\omega7$ (13·3, 9·1 and 17·4 %, respectively) and i-$C_{17:0}$ 3-OH (3·7, 7·8 and 2·8 %, respectively). Isoprenoid quinones were extracted and analysed by the method of Nakagawa & Yamasato (1993). The major lipoquinone was MK-6. The results of chemotaxonomic analyses support the affiliation of the sea water isolates to the family *Flavobacteriaceae*, all members of which are characterized by the presence of menaquinone 6 as the only or major respiratory quinone and the predominance of fatty acids $C_{15:0}$, i-$C_{15:0}$ and i-$C_{17:0}$ 3-OH (Bernardet *et al.*, 1996, 2002).

Gram-staining reaction, oxidase, catalase and alkaline phosphatase activities, degradation of agar, starch, casein, gelatin, cellulose (filter paper and CM-cellulose), chitin, DNA, Tweens 20, 40 and 80, urea and alginic acids, flexirubin production, growth at different temperatures, NaCl concentrations and pH, production of acid from carbohydrates, nitrate reduction and production of H_2S, indole and acetoin (Voges–Proskauer reaction) were tested according to the methods of Gerhardt *et al.* (1994). Susceptibility to antibiotics was determined as described previously (Nedashkovskaya *et al.*, 2003). Gliding motility and spreading growth were determined by cultivation of strains on a medium that contained (l^{-1}): 1 g Bactopeptone (Difco), 1 g yeast extract (Difco), 15 g agar and half-strength artificial sea water. The results are summarized in Table 2 and in the species description (see below).

Strains KMM 3664^T, KMM 3669 and KMM 3915 have some traits in common with currently described *Cellulophaga* species, but can be differentiated from all of them by arabinose and raffinose oxidation and the absence of DNase production, alginate hydrolysis and susceptibility to carbenicillin. The strains do not grow at 37 °C, in contrast to *C. lytica* strains. Reduction of nitrates to nitrites is noted for the strains studied, but not for strains of *C. lytica* or *C. fucicola*. Strains KMM 3664^T, KMM 3669 and KMM 3915 differ from the type strains of *C. baltica* (NN015840^T) and *C. fucicola* (NN015860^T) by the absence of casein hydrolysis.

Based on the results of the polyphasic taxonomic analysis that is presented in this work, the new environmental isolates clearly represent a novel species in the genus *Cellulophaga*. We propose that strains KMM 3664^T, KMM 3669 and KMM 3915 should be placed in this genus as members of a novel species, *Cellulophaga pacifica* sp. nov.

Description of *Cellulophaga pacifica* sp. nov.

Cellulophaga pacifica (pa.ci′fi.ca. N.L. adj. *pacifica* still; referring to the Pacific Ocean, from which the organism was isolated).

Cells are Gram-negative, strictly aerobic, chemo-organotrophic, motile by gliding, asporogenic rods that are 0·5–0·7 μm wide and 2·7–5·3 μm long. Oxidase-, catalase- and alkaline phosphatase-positive. Colonies are circular, low convex, shiny with entire edges, weakly sunken into agar and 1–3 mm in diameter on marine agar 2216. Yellow, non-diffusible pigments are produced. No growth is observed without Na^+. Growth occurs at 1–8 % NaCl. pH range for growth is 5·5–10·0, with optimum growth at pH 7·5–8·5. Flexirubin pigments are absent. Growth is detected at 4 and 34 °C. Agar, gelatin, starch and Tweens 20, 40 and 80 are hydrolysed, but casein, cellulose (CM-cellulose and filter paper), alginic acids, chitin and DNA are not. Nitrate reduction is positive. Indole, acetoin (Voges–Proskauer reaction) and H_2S are not produced. Acid is formed from arabinose, cellobiose, galactose, glucose, lactose, maltose, raffinose, sucrose and xylose, but not from melibiose, rhamnose, sorbose, *N*-acetylglucosamine, adonitol, dulcitol, glycerol, inositol, sorbitol or mannitol. Strains are susceptible to carbenicillin, oleandomycin and lincomycin and resistant to kanamycin, benzylpenicillin, neomycin, tetracycline, gentamicin and polymyxin B.

Table 2. Phenotypic properties of *Cellulophaga pacifica* and other *Cellulophaga* species

Strains: 1, *C. pacifica* KMM 3664^T; 2, *C. pacifica* KMM 3669; 3, *C. pacifica* KMM 3915; 4, *C. lytica* ATCC 23178^T; 5, *C. algicola* ACAM 630^T; 6, *C. baltica* NN015840^T; 7, *C. fucicola* NN015860^T. Data are from Johansen *et al.* (1999), Bowman (2000) and this study. All strains tested were positive for the following characteristics: motion by gliding, respiratory metabolism, oxidase, catalase and alkaline phosphatase production, Na^+ requirement for growth, growth at 4 °C, growth at 6 % NaCl, hydrolysis of agar, gelatin and starch and susceptibility to lincomycin and oleandomycin. All strains were negative for the following characteristics: flexirubin pigments, growth at 10 % NaCl, hydrolysis of cellulose (CM-cellulose and filter paper) and chitin, production of H_2S, indole and acetoin (Voges–Proskauer reaction), acid from rhamnose, sorbose, *N*-acetylglucosamine, adonitol, dulcitol, glycerol, inositol and sorbitol and susceptibility to benzylpenicillin, gentamicin, kanamycin and neomycin.

Characteristic	1	2	3	4	5	6	7
Growth at							
8 % NaCl	−	+	+	−	−	−	−
37 °C	−	−	−	+	−	−	−
Hydrolysis of:							
Casein	−	−	−	−	−	+	+
Alginate	−	−	−	+	+	+	+
DNA	−	−	−	+	+	+	+
Tween 20	+	+	+	+	−	+	+
Tween 40	+	+	+	+	+	−	+
Tween 80	+	+	−	+	−	+	+
Nitrate reduction	+	+	+	−	+	+	−
Acid from:							
Arabinose	+	+	+	−	−	−	−
Cellobiose	+	+	+	+	−	−	+
Fucose	−	+	−	−	−	+	−
Galactose	+	+	+	+	−	−	−
Glucose	+	+	+	+	+	−	+
Lactose	+	+	+	+	+	−	−
Maltose	+	+	+	+	+	+	−
Mellibiose	−	−	−	−	+	−	−
Raffinose	+	+	+	−	−	−	−
Sucrose	+	+	+	−	−	+	−
Xylose	+	−	+	+	−	−	−
Mannitol	−	−	−	−	−	+	−
Susceptibility to:							
Ampicillin	−	+	+	−	−	−	−
Carbenicillin	+	+	+	−	−	−	−
Streptomycin	+	−	−	−	−	−	−
Tetracycline	−	−	−	−	+	−	−
DNA G+C content (mol%), as determined by:							
Thermal denaturation method	33·2	33·0	34·3	33·0	36·1	34·2	34·0
HPLC	31·6	32·0			33·4	34·6	

Predominant cellular fatty acids are i-$C_{15:0}$, i-$C_{15:1}$, $C_{15:0}$, $C_{16:1}\omega7$ and i-$C_{17:0}$ 3-OH. Major lipoquinone is MK-6. DNA G+C content is 32–34 mol% (T_m and HPLC).

The type strain is KMM 3664^T (=JCM 11735^T=LMG 21938^T). Isolated from sea water.

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References

Barbeyron, T., L'Haridon, S., Corre, E., Kloareg, B. & Potin, P. (2001). *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as

Zobellia uliginosa gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 985–997.

Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.

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.

Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. & Swings, J. (2002). Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int J Syst Evol Microbiol* **52**, 1551–1558.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, F. (1995). PHYLIP (Phylogeny Inference Package) version 3.57c. Seattle: University of Washington.

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.

Hiraishi, A. (1992). Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. *Lett Appl Microbiol* **15**, 210–213.

Johansen, J. E., Nielsen, P. & Sjøholm, C. (1999). Description of *Cellulophaga baltica* gen. nov., sp. nov. and *Cellulophaga fucicola* gen. nov., sp. nov. and reclassification of [*Cytophaga*] *lytica* to *Cellulophaga lytica* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 1231–1240.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Leisner, J. J., Vancanneyt, M., Lefebvre, K., Vandemeulebroecke, K., Hoste, B., Euras Vilalta, N., Rusul, G. & Swings, J. (2002). *Lactobacillus durianis* sp. nov., isolated from an acid-fermented condiment (tempoyak) in Malaysia. *Int J Syst Evol Microbiol* **52**, 927–931.

Lewin, R. A. (1969). A classification of flexibacteria. *J Gen Microbiol* **58**, 189–206.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

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

Mesbah, M., Premachandran, U. & Whitman, W. B. (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., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003). *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga–Flavobacterium–Bacteroides*. *Int J Syst Evol Microbiol* **53**, 81–85.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Reichenbach, H. (1989). Genus I. *Cytophaga* Winogradsky 1929, 577,[AL] emend. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2015–2050. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. C. Holt. Baltimore: Williams & Wilkins.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (2001). Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1639–1652.

Svetashev, V. I., Vysotskii, M. V., Ivanova, E. P. & Mikhailov, V. V. (1995). Cellular fatty acids of *Alteromonas* species. *Syst Appl Microbiol* **18**, 37–43.

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.

Van de Peer, Y., De Rijk, P., Wuyts, J., Winkelmans, T. & De Wachter, R. (2000). The European small subunit ribosomal RNA database. *Nucleic Acids Res* **28**, 175–176.

Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 241–245. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Greene Publishing and Wiley.

ZoBell, C. E. & Upham, H. C. (1944). A list of marine bacteria including descriptions of sixty new species. *Bull Scripps Inst Oceanogr Univ Calif* **5**, 239–292.