Cellulophaga geojensis sp. nov., a member of the family *Flavobacteriaceae* isolated from marine sand

Sooyeon Park,¹ Ki-Hoon Oh,¹ Soo-Young Lee,¹ Tae-Kwang Oh¹
and Jung-Hoon Yoon²

Correspondence
Jung-Hoon Yoon
jhyoon69@skku.edu

¹Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yuseong, Daejeon, Republic of Korea

²Department of Food Science and Biotechnology, Sungkyunkwan University, Jangan-gu, Suwon, Republic of Korea

A Gram-stain-negative, aerobic, non-flagellated, non-spore-forming, motile (by gliding) bacterial strain, designated M-M6^T, was isolated from marine sand of Geoje island, Korea. Strain M-M6^T grew optimally at 25 °C, at pH 7.0–8.0 and in the presence of 2 % (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain M-M6^T fell within the clade comprising *Cellulophaga* species, forming a coherent cluster with *Cellulophaga lytica* ATCC 23178^T and *Cellulophaga fucicola* NN015860^T, with which it shared 16S rRNA gene sequence similarities of 98.1 and 98.2 %, respectively. Sequence similarities between strain M-M6^T and the type strains of other recognized *Cellulophaga* species were in the range 92.4–93.8 %. Strain M-M6^T contained MK-6 as the predominant menaquinone and iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH, and C_{16:1} ω7c and/or iso-C_{15:0} 2-OH as the major fatty acids. The major polar lipids detected in strain M-M6^T and the type strains of *C. lytica* and *C. fucicola* were two unidentified lipids, one unidentified aminolipid and one unidentified aminophospholipid. The DNA G + C content of strain M-M6^T was 35.4 mol%. Levels of DNA–DNA relatedness between strain M-M6^T and *C. lytica* JCM 8516^T and *C. fucicola* JCM 21778^T were 33 and 35 %, respectively. Differential phenotypic properties and phylogenetic and genetic distinctiveness distinguished strain M-M6^T from all recognized *Cellulophaga* species. On the basis of the data presented, strain M-M6^T is considered to represent a novel species of the genus *Cellulophaga*, for which the name *Cellulophaga geojensis* sp. nov. is proposed. The type strain is M-M6^T (=KCTC 23498^T=CCUG 60801^T).

During screening of polysaccharide-degrading micro-organisms from marine environments in the South Sea, Korea, many bacterial strains were isolated and characterized. One of these isolates, designated M-M6^T, which was isolated from marine sand, is described in this study. Comparative 16S rRNA gene sequence analysis showed that this novel strain is phylogenetically most closely related to members of the genus *Cellulophaga*. The genus *Cellulophaga*, a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes* (Bernardet & Nakagawa, 2006), was created by Johansen *et al.* (1999) with the reclassification of [*Cytophaga*] *lytica* (Lewin, 1969) as *Cellulophaga lytica* and the description of two novel species, *Cellulophaga baltica* and *Cellulophaga fucicola*. At the time of writing, the genus *Cellulophaga* comprised six recognized species,

C. lytica, *C. baltica* and *C. fucicola* (Johansen *et al.*, 1999), *Cellulophaga algicola* (Bowman, 2000), *Cellulophaga pacifica* (Nedashkovskaya *et al.*, 2004) and *Cellulophaga tyrosin-oxydans* (Kahng *et al.*, 2009). All members of the genus *Cellulophaga* have been isolated from marine habitats (Johansen *et al.*, 1999; Bowman, 2000; Nedashkovskaya *et al.*, 2004; Kahng *et al.*, 2009). The aim of the present study was to determine the exact taxonomic position of strain M-M6^T by using a polyphasic characterization.

Strain M-M6^T was isolated by the dilution plating technique on marine agar 2216 (MA; Difco) at 25 °C and was cultivated routinely on MA at 25 °C. *C. fucicola* JCM 21778^T and *C. lytica* JCM 8516^T, which were used as reference strains for DNA–DNA hybridization, fatty acid and polar lipid analyses, and a number of other phenotypic tests, were obtained from the Japan Collection of Microorganisms (JCM), Saitama, Japan. Cell morphology was examined by light microscopy (DP70; Olympus) and transmission electron microscopy (CM-20; Philips). The

Abbreviation: CMC, carboxymethylcellulose.

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain M-M6^T is HQ596527.

A supplementary figure is available with the online version of this paper.

latter technique was also used to assess the presence of flagellation on cells from an exponentially growing MA culture. For this, cells were negatively stained with 1% (w/v) phosphotungstic acid and grids were examined after being air-dried. Gliding motility was investigated as described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (1029; Forma, N₂/CO₂/H₂, 86:7:7%) on MA and on MA supplemented with potassium nitrate (0.1%, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Growth at 4, 15, 20, 25, 30, 37, 40 and 45 °C was measured on MA. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0% (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded and that 0.45% (w/v) MgCl₂·6H₂O was added. Growth in the presence of 2.0–10.0% (w/v) NaCl (final concentration, increments of 1.0%) was measured in marine broth 2216 (MB; Difco). The pH range for growth was determined in MB adjusted to pH 4.5–9.5 (increments of 0.5 pH units) by using sodium acetate/acetic acid and Na₂CO₃. pH was verified after autoclaving. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, hypoxanthine, xanthine, starch and L-tyrosine were performed on MA, by using the substrate concentrations described by Cowan & Steel (1965). Nitrate reduction and hydrolysis of aesculin, gelatin, Tweens (20, 40, 60 and 80) and urea were investigated as described by Lányi (1987) with the modification that artificial seawater was used for preparation of media. Degradation of carboxymethylcellulose (CMC) and carrageenan was tested on basal medium agar [per litre artificial seawater: 12 g gellan gum, 1 g yeast extract, 0.5 g NH₄Cl, 50 ml 1 M Tris/HCl (pH 7.4) and 5 g low-melting-point agarose] containing 0.5% (w/v) CMC (Sigma) and *ι*-carrageenan (Sigma), respectively. Degradation activities of CMC and carrageenan were determined by flooding the agar surface with Congo red solution and 10× diluted Lugol's iodine solution, respectively. The artificial seawater contained (per litre distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Acid production from carbohydrates was determined according to Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981), by using supplementation with 2% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1% (v/v) vitamin solution (Staley, 1968). H₂S production was tested as described by Bruns *et al.* (2001). Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs (Advantec) containing the following (µg per disc unless otherwise stated): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15),

neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), polymyxin B (100 U), streptomycin (50) and tetracycline (30). Enzyme activities and additional

Table 1. Differential phenotypic characteristics of strain M-M6^T and the type strains of two phylogenetically related *Cellulophaga* species

Strains: 1, M-M6^T; 2, *C. lytica* JCM 8516^T; 3, *C. fucicola* JCM 21778^T. Data are from this study unless otherwise indicated. All strains are positive for: gliding motility; activity of catalase and oxidase; hydrolysis of agar, aesculin, carrageenan, CMC, gelatin, starch, L-tyrosine and Tweens (20, 40, 60 and 80); utilization of cellobiose, D-galactose, D-glucose, maltose, salicin and trehalose; acid production from cellobiose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, melibiose, trehalose and D-xylose; activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase; and susceptibility to chloramphenicol, lincomycin and oleandomycin. All strains are negative for: Gram-staining; nitrate reduction; production of H₂S; production of flexirubin-type pigments; hydrolysis of hypoxanthine, urea and xanthine; utilization of L-arabinose, acetate, benzoate, citrate, formate, L-malate, pyruvate and succinate; acid production from L-arabinose, melezitose, myo-inositol, raffinose, L-rhamnose, D-ribose and D-sorbitol; activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase and α-fucosidase; and susceptibility to ampicillin, gentamicin, kanamycin, neomycin, penicillin G, polymyxin B, streptomycin and tetracycline.

Characteristic	1	2	3
Hydrolysis of casein	+	–	+
Utilization of:			
D-Fructose	+	–	–
L-Glutamate	+	–	–
D-Mannose	+	–	–
Sucrose	+	–	–
D-Xylose	+	–	–
Acid production from:			
D-Fructose	–	+	+
Sucrose	+	+	–
Enzyme activity (API ZYM)			
Cystine arylamidase	+	+	–
β-Galactosidase	+	+	–
α-Glucosidase	–	–	+
β-Glucosidase	–	–	+
N-Acetyl-β-glucosaminidase	+	–	+
α-Mannosidase	–	–	+
Susceptibility to:			
Carbenicillin	+	+	–
Cephalothin	–	+	–
Novobiocin	–	–	+
DNA G + C content (mol%)*	35.4	33.0	32.4

*Data for the two reference strains taken from Reichenbach (1989) and Johansen *et al.* (1999).

biochemical tests were performed by using the API ZYM and 20E systems (bioMérieux) with incubation at 25 °C for 8 h and 3 days, respectively, according to the manufacturer's instructions. Morphological, cultural, physiological and biochemical characteristics of strain M-M6^T are given in the species description below and in Table 1.

Cell biomass of strain M-M6^T for DNA extraction and for analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 3 days in MB at 25 °C. Chromosomal DNA was extracted and purified by the procedure described by Yoon *et al.* (1996), with the modification that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR by using two universal primers as described previously (Yoon *et al.*, 1998) and the PCR products were purified by using a QIAquick PCR purification kit (Qiagen). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The almost-complete 16S rRNA gene sequence of strain M-M6^T determined in this study comprised 1441 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain M-M6^T fell within the clade comprising *Cellulophaga* species, clustering coherently with the type strains of *C. lytica* and *C. fucicola* (Fig. 1). The relationships among strain M-M6^T, *C. lytica* ATCC 23178^T and *C. fucicola* NN015860^T were also maintained in the trees constructed with the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain M-M6^T exhibited 16S rRNA gene sequence similarity values of 98.1 and 98.2 % to *C. lytica* ATCC 23178^T and *C. fucicola*

NN015860^T, respectively, and 92.4–93.8 % to the type strains of other recognized *Cellulophaga* species.

Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed by using reversed-phase HPLC with a YMC ODS-A (250 × 4.6 mm) column. The predominant isoprenoid quinone detected in strain M-M6^T was menaquinone-6 (MK-6), which is in line with data for the genus *Cellulophaga* (Nedashkovskaya *et al.*, 2004; Kahng *et al.*, 2009) and all members of the family *Flavobacteriaceae* (Bernardet & Nakagawa, 2006). For cellular fatty acid analysis, cell mass of strain M-M6^T, *C. lytica* JCM 8516^T and *C. fucicola* JCM 21778^T was harvested from MA plates after incubation for 3 days at 25 °C. Fatty acids were saponified, methylated and extracted by using the standard MIDI protocol (Sherlock Microbial Identification System, version 4.0). The mixture of fatty acids was separated by GC (Hewlett Packard 6890) and components were identified by using the TSBA40 database of the Microbial Identification System (Sasser, 1990). The cellular fatty acid profile of strain M-M6^T is shown in Table 2, together with those of *C. lytica* JCM 8516^T and *C. fucicola* JCM 21778^T. The major fatty acids (>10 % of the total) found in strain M-M6^T were iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH, and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (Table 2). The overall fatty acid profile of strain M-M6^T was similar to those of *C. lytica* JCM 8516^T and *C. fucicola* JCM 21778^T, but the three strains could be differentiated based on differences in the respective proportions of some fatty acids (Table 2). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with molybdophosphoric

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain M-M6^T, the type strains of recognized *Cellulophaga* species and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >70 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Bacteroides fragilis* ATCC 25285^T (GenBank accession no. X83935) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of strain M-M6^T and the type strains of two phylogenetically related *Cellulophaga* species

Strains: 1, M-M6^T; 2, *C. lytica* JCM 8516^T; 3, *C. fucicola* JCM 21778^T. All data are from this study. Fatty acids that represented <1.0 % in all strains were omitted. tr, Trace (<0.5 %); –, not detected.

Fatty acid	1	2	3
Straight-chain			
C _{14:0}	1.2	0.6	1.5
C _{15:0}	7.4	7.4	10.2
C _{16:0}	3.2	1.0	tr
Branched			
iso-C _{14:0}	1.1	1.2	0.8
iso-C _{15:0}	14.9	32.0	24.3
iso-C _{16:0}	0.9	1.1	tr
iso-C _{15:1} G*	14.0	14.4	15.3
anteiso-C _{15:0}	1.5	3.8	3.3
iso-C _{17:1} ω9c	–	1.8	–
Unsaturated			
C _{15:1} ω6c	1.7	1.2	3.2
Hydroxy			
C _{15:0} 3-OH	0.9	0.8	1.9
C _{16:0} 3-OH	5.4	1.3	1.5
iso-C _{15:0} 3-OH	9.2	9.5	14.4
iso-C _{16:0} 3-OH	8.9	3.2	1.8
iso-C _{17:0} 3-OH	11.5	10.1	6.2
Summed features†			
3	10.6	5.7	8.7
5	1.7	–	–
Unknown fatty acid‡			
ECL 13.565	2.4	2.9	2.6

*Double bond position indicated by a capital letter is unknown.

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprised C_{16:1} ω7c and/or iso-C_{15:0} 2-OH; summed feature 5 comprised C_{18:2} ω6,9c and/or anteiso-C_{18:0}.

‡ECL, Equivalent chain-length.

acid, molybdenum blue, ninhydrin and α-naphthol reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987) and Dragendorff's reagent (Sigma). The polar lipid profiles of strain M-M6^T and the type strains of *C. lytica*, the type species of the genus, and *C. fucicola* were almost identical, in that large amounts of two unidentified lipids, one unidentified aminolipid and one unidentified amino-phospholipid are present (see Fig. S1 in IJSEM Online). The DNA G+C content was determined according to the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC [with a YMC ODS-A (250 × 4.6 mm) column]. The nucleotides were eluted with a mixture of 0.55 M NH₄H₂PO₄ (pH 4.0) and acetonitrile (40:1, v/v) using a flow rate of 1 ml min⁻¹ at room temperature and were detected by UV absorbance at 270 nm. The DNA G+C content of strain M-M6^T was 35.4 mol%, a

value slightly higher than those reported for members of the genus *Cellulophaga* (Kahng *et al.*, 2009). Hence, it is reasonable to classify strain M-M6^T as a member of the genus *Cellulophaga*, as shown by phylogenetic inference and the absence of differential chemotaxonomic properties from recognized *Cellulophaga* species (Johansen *et al.*, 1999; Bowman, 2000; Nedashkovskaya *et al.*, 2004; Kahng *et al.*, 2009).

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values are quoted as DNA–DNA relatedness values. Strain M-M6^T exhibited mean levels of DNA–DNA relatedness of 33 and 35 % to *C. lytica* JCM 8516^T and *C. fucicola* JCM 21778^T, respectively. These values indicate that strain M-M6^T represents a genomic species that is distinct from these two *Cellulophaga* species (Wayne *et al.*, 1987). Strain M-M6^T was clearly distinguishable from *C. lytica* JCM 8516^T and *C. fucicola* JCM 21778^T based on differences in several phenotypic characteristics (Table 1). Phylogenetic and genetic distinctiveness together with differential phenotypic properties are sufficient to allocate strain M-M6^T to a species that is separate from recognized *Cellulophaga* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain M-M6^T is considered to represent a novel species of the genus *Cellulophaga*, for which the name *Cellulophaga geojensis* sp. nov. is proposed.

Description of *Cellulophaga geojensis* sp. nov.

Cellulophaga geojensis (ge.o.jen'sis. N.L. fem. adj. *geojensis* pertaining to Geoje, the Korean island from where the type strain was isolated).

Cells are Gram-stain-negative, non-spore-forming, non-flagellated and rod-shaped (0.5–0.8 µm in diameter and 2.0–6.0 µm in length). Motile by gliding. Colonies on MA are circular, slightly convex, smooth, glistening, yellow and 1.0–2.0 mm in diameter after incubation for 3 days at 25 °C. Optimal growth occurs at 25 °C; growth occurs at 4 and 40 °C, but not at 45 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs weakly at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2 % (w/v) NaCl; growth occurs with 0–7.0 % (w/v) NaCl. Mg²⁺ ions are required for growth. Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Flexirubin-type pigments are not produced. Catalase- and oxidase-positive. Nitrate reduction is negative. Agar, aesculin, casein, carrageenan, CMC, gelatin, starch, L-tyrosine, and Tweens 20, 40, 60 and 80 are hydrolysed, but hypoxanthine, urea and xanthine are not. Cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, salicin, sucrose, trehalose, D-xylose and L-glutamate are utilized as sole carbon and energy sources,

but L-arabinose, acetate, benzoate, citrate, formate, L-malate, pyruvate and succinate are not. Acid is produced from cellobiose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, melibiose, sucrose, trehalose and D-xylose, but not from L-arabinose, D-ribose, D-fructose, myo-inositol, melezitose, raffinose, L-rhamnose or D-sorbitol. In assays with the API ZYM and API 20E systems, positive for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase and N-acetyl- β -glucosaminidase, but negative for activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lipase (C14), trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase. The predominant menaquinone is MK-6. The major fatty acids (>10 % of the total) are iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH, and C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH. The major polar lipids are two unidentified lipids, one unidentified aminolipid and one unidentified aminophospholipid. Other phenotypic characteristics are given in Table 1.

The type strain, M-M6^T (=KCTC 23498^T=CCUG 60801^T), was isolated from marine sand of Geoje island, Korea. The DNA G+C content of the type strain is 35.4 mol% (determined by HPLC).

Acknowledgements

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications (grant 11-2008-00-002-00) and the Program for Collection, Management and Utilization of Biological Resources (grant M10867010003) from the Ministry of Education, Science and Technology (MEST) of the Republic of Korea.

References

- Baumann, P. & Baumann, L. (1981). The marine Gram-negative eubacteria: genera *Photobacterium*, *Beneckeia*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes*, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- 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.
- 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.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* 51, 1997–2006.
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by nonsulfur purple bacteria. *J Cell Comp Physiol* 49, 25–68.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- 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.
- 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.
- Kahng, H.-Y., Chung, B. S., Lee, D.-H., Jung, J. S., Park, J. H. & Jeon, C. O. (2009). *Cellulophaga tyrosinoydians* sp. nov., a tyrosinase-producing bacterium isolated from seawater. *Int J Syst Evol Microbiol* 59, 654–657.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–207.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 19, 1–67.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* 85, 1183–1184.
- Lewin, R. A. (1969). A classification of flexibacteria. *J Gen Microbiol* 58, 189–206.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & 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., Suzuki, M., Lysenko, A. M., Snauwaert, C., Vancanneyt, M., Swings, J., Vysotskii, M. V. & Mikhailov, V. V. (2004). *Cellulophaga pacifica* sp. nov. *Int J Syst Evol Microbiol* 54, 609–613.
- Reichenbach, H. (1989). Genus I. *Cytophaga* Winogradsky 1929, 557^{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. G. Holt. Baltimore: Williams & Wilkins.
- Reichenbach, H. (1992). The order Cytophagales. In *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd edn, vol. 4, pp. 3631–3675. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* 44, 846–849.
- Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* 95, 1921–1942.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 25, 125–128.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* 46, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioideis* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* 48, 187–194.
- Yoon, J.-H., Kim, I.-G., Shin, D.-Y., Kang, K. H. & Park, Y.-H. (2003). *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int J Syst Evol Microbiol* 53, 53–57.