Flavobacterium xinjiangense sp. nov. and *Flavobacterium omnivorum* sp. nov., novel psychrophiles from the China No. 1 glacier

Fei Zhu, Shan Wang and Peijin Zhou

Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, PR China

Correspondence

Peijin Zhou

zhou@sun.im.ac.cn

Two novel psychrophilic bacterial strains (ZF-6^T and ZF-8^T) were isolated from the China No. 1 glacier. Polyphasic taxonomy using physiological and biochemical properties and phylogenetic analysis based on 16S rRNA gene sequences showed that the two isolates belonged to the genus *Flavobacterium*, and that they were distinct from each other and also from the known species of this genus. Strains ZF-6^T and ZF-8^T are Gram-negative and both have an optimal growth temperature of 11 °C. Strain ZF-6^T is able to grow at 0–20 °C, the G + C content of its genomic DNA is 34.4 mol% and the major fatty acids of ZF-6^T are C_{16:1}ω7c (17.7 %) and C_{15:1}ω6c (12.7 %). Strain ZF-8^T showed a strong ability to degrade organic macromolecules such as starch, CM-cellulose, pectin and chitin. Its DNA G + C content is 35.1 mol%, and the major fatty acids are C_{16:1}ω7c (18.2 %) and C_{15:0} (9.9 %). Phylogenetic analysis based on 16S rDNA sequences indicated that ZF-6^T and ZF-8^T belong to the genus *Flavobacterium* and represent two novel species. DNA–DNA hybridization also supported the status of the two new isolates. The names *Flavobacterium xinjiangense* sp. nov. (type strain, ZF-6^T = AS 1.2749^T = JCM 11314^T) and *Flavobacterium omnivorum* sp. nov. (type strain, ZF-8^T = AS 1.2747^T = JCM 11313^T) are proposed for the two new isolates.

Cold-adapted micro-organisms are divided into two categories: obligately psychrophilic micro-organisms (psychrophiles) and facultatively psychrophilic micro-organisms (psychrotolerant organisms). A psychrophile is capable of growing at or below 0 °C, but is unable to grow above 20 °C. A psychrotolerant organism, whilst capable of growth at 0 °C, can grow well above 20 °C (Morita, 1975). As 80 % of the biosphere has a temperature that remains permanently below 5 °C, cold-adapted micro-organisms are widely distributed in nature (Margesin & Schinner, 1994). Most of the psychrophiles that have been characterized so far originate from the Antarctic. Psychrophiles can also be found in permanently cold environments such as fresh and marine water, polar and high alpine soils and water, glaciers and frozen or chilled foodstuffs (Margesin & Schinner, 1994). The glacier is a relatively simple and closed ecosystem with a special biotic community, containing various psychrophilic and psychrotolerant organisms (Christner *et al.*, 2000). In China, there are more than 46 000 glaciers, with a total area of >59 000 km². China No. 1 glacier has an area of 1.74 km² and is located in Xinjiang province, North-West

China. Cold-adapted micro-organisms that inhabit these environments have not been well studied.

The genus *Flavobacterium* was proposed by Frankland in 1889 (Bergey *et al.*, 1923); the description of the genus has been emended several times since then. Bernardet *et al.* (1996) proposed an emendment to the description, which states that the genus *Flavobacterium* represents predominantly gliding, pigmented bacteria and has menaquinone-6 as the primary respiratory quinone. Species of the genus *Flavobacterium* have DNA G + C contents of 32–37 mol%. Recently, several psychrophilic species of *Flavobacterium* have been reported (McCammon & Bowman, 2000; Humphry *et al.*, 2001). In this study, we describe two newly isolated psychrophilic bacterial strains from the China No. 1 glacier. Morphological, physiological and chemotaxonomic studies indicated that the isolates should be placed in the genus *Flavobacterium*. Systematic studies on 16S rDNA similarity and DNA–DNA reassociation further distinguished the two isolates from known species in the genus *Flavobacterium*. The names *Flavobacterium xinjiangense* sp. nov. and *Flavobacterium omnivorum* sp. nov. are proposed for these isolates.

Samples of frozen soil from the China No. 1 glacier (Xinjiang province) were collected in September 1999. Samples were stored for 2 days in an ice chest until they

Published online ahead of print on 1 November 2002 as DOI 10.1099/ijs.0.02310-0.

The GenBank accession numbers of the 16S rDNA sequences of ZF-6^T and ZF-8^T are AF433173 and AF433174, respectively.

could be processed in the laboratory. The frozen soil was suspended in an equal volume of liquid PYG medium and incubated for 1–2 days at 4 °C. Two millilitres of the suspension was used to inoculate PYG agar plates. The plates were incubated at 4 °C for 2 weeks. Strains ZF-6^T and ZF-8^T were obtained by selection of colonies that did not grow at 20 °C or above. PYG medium contained (l⁻¹): 5.0 g polypeptone, 5.0 g tryptone, 10.0 g yeast extract, 10.0 g glucose and 40 ml salt solution. The salt solution, pH 7.2, contained (l⁻¹): 0.2 g CaCl₂, 0.4 g MgSO₄·7H₂O, 1.0 g K₂HPO₄, 1.0 g KH₂PO₄, 10.0 g NaHCO₃ and 2.0 g NaCl. The reference strains, *Flavobacterium gillisiae* ACAM 601^T, *Flavobacterium xanthum* ACAM 81^T, *Flavobacterium columnare* ATCC 23463^T and *Flavobacterium frigidarium* ATCC 700810^T, were cultivated as recommended (Bernardet *et al.*, 1996; McCammon & Bowman, 2000; Humphry *et al.*, 2001). The size and morphology of cells grown in PYG medium at 11 °C for 16–24 h were measured and observed by transmission electron microscopy. Cells were negatively stained with 1% (w/v) phosphotungstic acid; after air-drying, the grids were examined by using a model H-600 transmission electron microscope (Hitachi). Motility was determined by phase-contrast microscopy, using the hanging-drop technique (Skerman, 1967). For determination of gliding motility, strains were grown for 16–24 h at 11 °C on various dilute PYG media (solidified with 1.3% agar, w/v), prepared as a thin layer on ethanol-rinsed microscope slides. Following incubation, the edges of the colonies were observed by using phase microscopy.

General physiological tests were performed as described by Smibert & Krieg (1981). Utilization of carbon and energy sources was investigated by the use of basal medium containing (l⁻¹): 0.1 g yeast extract (Oxoid), 1 g (NH₄)₂HPO₄ and 40 ml salt solution (pH 7.2). The medium was solidified with 1.3% (w/v) purified agar (Oxoid). Carbon substrates were added at a concentration of 0.5% (w/v). Testing for oxidative and fermentative acid production from carbohydrates was performed by using Leifson's medium (Leifson, 1963). Chitin hydrolysis was tested as described by Hsu & Lockwood (1975). The detection of flexirubin-type pigments using 20% KOH and of extracellular glycans using the Congo red absorption test was performed according to McCammon & Bowman (2000). Growth temperature was determined using a TN3F temperature-gradient incubator (Advantec). For quantitative analysis of cellular fatty acids, a loop of cell mass grown on PYG agar at 11 °C for 2 days was harvested. Fatty acid methyl esters were prepared and identified using standard methods (Stackebrandt *et al.*, 1995), and the results were compared to the database of fatty acids in the MIDI Sherlock Microbial Identification system (Microbial ID).

Genomic DNA was extracted and purified as described by Sambrook *et al.* (1989). The DNA G + C content was determined by the thermal denaturation method (Sly *et al.*, 1986) with a Shimadzu UV-1206 spectrophotometer equipped with a TB-85 thermostat. DNA–DNA hybridization was

carried out as described by Tindall *et al.* (1984). DNA fragments were labelled with [α -³²P]dATP, following the instructions provided with the Nick Translation kit (Promega). The 16S rRNA genes of strains ZF-6^T and ZF-8^T were amplified by PCR using the Promega *Taq* kit with primers 43F and 1541R (Liu *et al.*, 2000). Purified PCR products were ligated to the pGEM-T vector (Promega) and cloned according to the manufacturer's instructions. Sequencing reactions were carried out using the ABI PRISM BigDye Primer cycle sequencing kit, and sequencing was performed on an ABI 373S DNA sequencer (both Applied Biosystems). Almost-complete 16S rDNA nucleotide sequences of ZF-6^T and ZF-8^T were determined (1466 nt for both). The 16S rDNA sequences of ZF-6^T and ZF-8^T were aligned with reference sequences from GenBank by using the multiple sequence alignment program CLUSTAL W (Thompson *et al.*, 1994). After removing non-base characters and ambiguous bases from the reference sequences, 1177 nt was used for DNA similarity calculation and phylogenetic tree construction. Evolutionary distance matrices were calculated by using the algorithm of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1993). A phylogenetic tree based on K_{nuc} was constructed using the neighbour-joining method with the Kimura two-parameter model in TREECON for Windows (Van de Peer & De Wachter, 1994).

Cells of strains ZF-6^T and ZF-8^T were strictly aerobic, Gram-negative and rod-shaped with rounded ends (Fig. 1). Colonies on PYG agar were circular, convex with entire margins and a smooth appearance. The optimal temperature for growth was 11 °C; growth occurred at 0 °C, but not at 20 °C. Motility of the strains was not detected by phase-contrast microscopy in semi-solid medium culture. Gliding motility was not observed. The two strains were not able to grow on sea-water agar. Flexirubin pigments were absent. Colonies of ZF-6^T on PYG medium were pale yellow, while those of ZF-8^T were orange. The other characteristic physiological differences between ZF-6^T and ZF-8^T are summarized in Table 1.

Strains ZF-6^T and ZF-8^T possessed similar fatty acid profiles, shown in Table 2. The most abundant fatty acid of ZF-6^T and ZF-8^T was C_{16:1}ω7c, in line with other psychrophilic *Flavobacterium* species (Bernadet *et al.*, 1996; McCammon & Bowman, 2000; Humphry *et al.*, 2001). In addition, ZF-6^T and ZF-8^T did not seem to produce i-C_{17:1}ω7c, i-C_{17:1}ω5c or C_{17:0}, which have commonly been found in the other psychrophilic *Flavobacterium* species (fatty acids representing <1% were not considered). There were abundant monounsaturated and branched fatty acids in the membranes of ZF-6^T and ZF-8^T, which is consistent with other psychrophilic *Flavobacterium* species. Such fatty acids contribute to the maintenance of membrane fluidity at low temperature (Russell & Hamamoto, 1998).

Comparative studies indicated that the 16S rDNA sequence of ZF-6^T shared 90.5–96.8% similarity with other *Flavobacterium* 16S rDNA sequences, with the most similar

Fig. 1. Transmission electron microphotographs of ZF-6^T (top) and ZF-8^T (bottom). Bars, 1 μm.

being that of *F. gillisiae* (96.8%). The 16S rRNA gene sequence of ZF-8^T was 92.0–97.3% similar to those of other *Flavobacterium* species, with the most similar being that of *F. columnare* (97.3%). ZF-6^T and ZF-8^T shared 96.6% similarity with each other. The phylogenetic tree clearly indicated that ZF-6^T and ZF-8^T should be included in the genus *Flavobacterium* (Fig. 2); they formed two distinct branches within the *Flavobacterium* clade, which were supported by bootstrap resampling values of 69 and 56%, respectively.

The DNA G + C contents of strains ZF-6^T and ZF-8^T were 34.4 and 35.1 mol%, respectively, which fell within the range expected for *Flavobacterium* species (32–37 mol%). These data were compatible with the assignment of the strains to the genus *Flavobacterium*.

The high sequence similarity of the new isolates among themselves and to some other *Flavobacterium* species

Table 1. Characteristics that differentiate ZF-6^T and ZF-8^T from other psychrophilic *Flavobacterium* species

Taxa: 1, ZF-6^T; 2, ZF-8^T (data for taxa 1–2 from this study); 3, *F. gillisiae*; 4, *F. xanthum*; 5, *F. tegetincola*; 6, *F. psychrophilum* [data for taxa 3–6 from McCammon & Bowman (2000)]; 7, *F. hibernum* [data from McCammon *et al.* (1998)]; 8, *F. frigidarium* [data from Humphry *et al.* (2001)]. +, Positive; (+), positive, weak or delayed response; –, negative; v, variable.

Characteristic	1	2	3	4	5	6	7	8
Growth on:								
Sea-water agar	–	–	+	+	+	–	–	+
Nutrient agar	(+)	+	+	+	+	–	+	+
Trypticase soy agar	–	+	+	+	+	–	+	+
Growth at 20 °C	–	–	+	+	+	–	+	+
Gliding motility	–	–	–	–	+	(+)	+	–
Flexirubin pigment	–	–	–	–	–	+	+	–
Glucose utilization	+	+	+	+	+	–	+	+
Acid from carbohydrates	–	–	+	+	+	–	+	–
Degradation of:								
Gelatin	+	–	–	+	–	+	+	+
Casein	+	+	+	+	–	+	+	+
Starch	–	+	+	+	–	–	+	–
CM-cellulose	–	+	–	–	–	–	–	–
Pectin	–	+	–	–	–	–	–	–
Chitin	+	+	+	–	–	–	–	–
Aesculin	+	+	+	+	–	–	+	+
DNA	–	–	–	–	–	(+)	+	–
Tyrosine	–	–	–	–	–	v	+	–
Precipitate on egg-yolk medium	–	–	–	–	–	+	–	–
Oxidase	+	+	–	+	–	–	+	+
ONPG hydrolysis	–	+	–	–	–	–	+	–
H ₂ S production	+	–	–	+	–	–	–	–
Nitrate reduction	–	+	–	+	–	–	+	–
G + C content (mol%)	34	35	32	36	32	35	36	35

necessitated DNA–DNA hybridization tests. The genomic DNA of ZF-6^T was 11.4, 19.3, 29.5 and 25.3% similar to that of *F. gillisiae* ACAM 601^T, *F. xanthum* ACAM 81^T, *F. frigidarium* ATCC 700810^T and ZF-8^T, respectively. The genomic DNA of ZF-8^T was 7.9, 14.6, 21.6, 28.2 and 13.6% similar to that of *F. gillisiae* ACAM 601^T, *F. xanthum* ACAM 81^T, *F. columnare* ATCC 23463^T, *F. frigidarium* ATCC 700810^T and ZF-6^T, respectively. Analysis of the results indicated that DNA similarities between ZF-6^T, ZF-8^T and other related *Flavobacterium* species were clearly below 70% (which is considered to be the threshold value for the delineation of species).

On the basis of phenotypic, genotypic and phylogenetic data, it is clear that ZF-6^T and ZF-8^T are members of the genus *Flavobacterium* and represent two distinct and novel species, for which the names *Flavobacterium xinjiangense* sp. nov. and *Flavobacterium omnivorum* sp. nov. are proposed. The phenotypic characteristics that differentiate these species from other psychrophilic species in the genus *Flavobacterium* are given in Table 1.

Table 2. Whole-cell fatty acid profiles of ZF-6^T, ZF-8^T and related strains

Taxa: 1, ZF-6^T; 2, ZF-8^T (data for taxa 1–2 from this study); 3, *F. gillisiae*; 4, *F. tegetincola*; 5, *F. xanthum* [data for taxa 3–5 from McCammon & Bowman (2000)]; 6, *F. frigidarium* [data from Humphry *et al.* (2001)].

Fatty acid	1	2	3	4	5	6
i-C _{13:0}				0.2		
a-C _{13:0}				0.3		
i-C _{14:1ω9c}			0.2			
C _{14:0}			0.4	0.5	0.4	1.6
i-C _{14:0}	2.1		0.7	0.8	0.3	3.66
C _{15:1ω6c}	12.7	4.4	21.5	1.8	7.9	
i-C _{15:1ω10c}	4.1	5.2	5.1	8.9	7.3	
a-C _{15:1ω10c}		2.4	1.3	5.7	2.2	
i-C _{15:0}	11.6	8.6	5.1	8.0	10.6	8.8
a-C _{15:0}	6.4	9.9	5.8	15.4	10.5	15.1
C _{15:0}	8.6	4.4	7.4	6.5	6.9	4.9
C _{16:1ω7c}	17.7	18.2	21.7	18.4	23.1	43.5
C _{16:1ω5c}			0.6	0.6	0.4	
i-C _{16:1ω6c}	2.4	5.6	3.2	3.8	0.5	
i-C _{16:0}		5.2	3.5	4.8	1.3	8.9
i-C _{16:0}						1.6
C _{16:0}			2.9	1.9	2.1	3.1
C _{17:1ω6c}	10.9	5.0	0.9	2.3	0.3	
i-C _{17:1ω9c}		5.9				
i-C _{17:1ω7c}			4.4	3.1	4.0	
i-C _{17:1ω5c}			1.0	3.5	2.6	
i-C _{17:0}			0.2		0.3	
a-C _{17:0}			1.5			
C _{18:0}			0.6			
i-C _{15:0} 3-OH	6.6	7.1	10.0	5.7	13.1	
a-C _{15:0} 3-OH			0.2	1.0	0.8	
C _{15:0} 3-OH	2.2		0.3	0.5	1.2	
i-C _{16:0} 3-OH	5.5	8.7	0.8	2.8	2.0	
a-C _{16:0} 3-OH			0.4	0.1	0.2	
C _{16:0} 2-OH						1
C _{16:0} 3-OH	4.4		0.4	1.1	1.7	1.7
i-C _{17:0} 3-OH	4.9	9.4	0.5	3.0	0.3	

Description of *Flavobacterium xinjiangense* sp. nov.

Flavobacterium xinjiangense (xin.jiang.en'se. N.L. neut. adj. *xinjiangense* pertaining to Xinjiang, an autonomous region in North-West China).

Gram-negative rods, 2.5–5 µm in length and 0.8 µm in width. Aerobic, psychrophilic, non-flagellated and non-gliding. Colonies are pale yellow, circular and smooth with entire edges. Optimal growth occurs at 11 °C; no growth occurs at 20 °C. No growth occurs in the presence of more than 3.5 % NaCl. Flexirubin pigments are not detected. Catalase- and oxidase-positive and strictly heteroorganotrophic. Metabolism is respiratory. H₂S is produced. Nitrate is not reduced. No precipitate is produced on egg-yolk agar.

Fig. 2. Phylogenetic tree based on 16S rDNA sequence divergence, showing the relationships between ZF-6^T, ZF-8^T and related reference strains. The tree was constructed using the neighbour-joining method with the Kimura two-parameter model. *Myroides odoratus* was used as the outgroup sequence. Numbers at branching points represent confidence levels from 1000 bootstrap resamplings, expressed as percentages. GenBank accession numbers are given in parentheses. Bar, 0.02 *K*_{nuc}.

The following biochemical tests are negative: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, ONPG hydrolysis, indole production, Voges–Proskauer reaction and Simmons' citrate test. Peptone and casamino acids, but not sodium nitrate, ammonium chloride or L-glutamate, serve as nitrogen sources. Does not produce any acid or gas from glucose, fructose, xylose, mannitol or maltose. Degrades casein, gelatin, aesculin and chitin, but not starch, Tween 80, agar, CM-cellulose, alginate, pectin, DNA, urea, uric acid or xanthine. The following are utilized as sole carbon sources: glucose, cellobiose, maltose, fructose, sucrose, arabinose and xylose. Sorbitol, sorbose, galactose, D-mannitol, raffinose, lactose, mannose, trehalose, glycerol, rhamnose, melibiose and inositol are not used as sole carbon sources. The major cellular fatty acids are C_{15:1ω6c} (12.7 %), i-C_{15:0} (11.6 %), C_{16:1ω7c} (17.7 %) and C_{17:1ω6c} (10.9 %). The G+C content of the DNA of the type strain is 34.4 mol%.

The type strain is ZF-6^T (=AS 1.2749^T=JCM 11314^T), isolated from frozen soil from the China No. 1 glacier.

Description of *Flavobacterium omnivorum* sp. nov.

Flavobacterium omnivorum (om.ni.vor'um. L. n. *omnis* everything; L. v. *vorare* to devour; N.L. neut. adj. *omnivorum*

eating everything, referring to the ability of the strain to degrade a wide range of macromolecules).

Gram-negative rods, 2–5 µm in length and 0.8 µm in width. Aerobic, psychrophilic, non-flagellated and non-gliding. Colonies are orange, circular and smooth with entire margins. Optimal growth occurs at 11 °C; no growth occurs at 20 °C. No growth occurs in the presence of more than 3.5 % NaCl. Flexirubin pigments are not detected. Catalase- and oxidase-positive and strictly heteroorganotrophic. Metabolism is respiratory. H₂S is not produced. Nitrate is reduced to nitrite. ONPG is hydrolysed. No precipitate is produced on egg-yolk agar. The following biochemical tests are negative: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, indole production, Voges–Proskauer reaction and Simmons' citrate test. Peptone, casamino acids, sodium nitrate and ammonium chloride, but not L-glutamate, serve as nitrogen sources. Does not produce any acid or gas from glucose, fructose, xylose, mannitol or maltose. Degrades casein, aesculin, chitin, starch, alginate, CM-cellulose and pectin, but not gelatin, Tween 80, agar, DNA, urea, uric acid or xanthine. The following are utilized as sole carbon sources: glucose, cellobiose, maltose, fructose, sorbose, sucrose, lactose, rhamnose, mannose, trehalose, melibiose and arabinose. Sorbitol, galactose, D-mannitol, raffinose, glycerol, inositol and xylose are not used as sole carbon sources. The major cellular fatty acids are C_{15:0} (9.9 %), C_{16:1ω7c} (18.2 %) and i-C_{17:0} 3-OH (9.4 %). The G + C content of the DNA of the type strain is 35.2 mol%.

The type strain is ZF-8^T (=AS 1.2749^T=JCM 11313^T), isolated from frozen soil from the China No. 1 glacier.

Acknowledgements

We thank Professor Shuang-Jiang Liu for his useful, critical discussion during the preparation of this manuscript.

References

- Bergey, D. H., Harrison, F. C., Breed, R. S., Hammer, B. W. & Huntoon, F. M. (1923). Genus II. *Flavobacterium* gen. nov. In *Bergey's Manual of Determinative Bacteriology*, pp. 97–117. Baltimore: Williams & Wilkins.
- 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.
- Christner, B. C., Mosley-Thompson, E., Thompson, L. G., Zagorodnov, V., Sandman, K. & Reeve, J. N. (2000). Recovery and identification of viable bacteria immured in glacial ice. *Icarus* **144**, 479–485.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.75c. University of Washington, Seattle, USA.
- Liu, H., Xu, Y., Ma, Y. & Zhou, P. (2000). Characterization of *Micrococcus antarcticus* sp. nov., a psychrophilic bacterium from Antarctica. *Int J Syst Evol Microbiol* **50**, 715–719.
- Hsu, S. C. & Lockwood, J. L. (1975). Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl Microbiol* **29**, 422–426.
- Humphry, D. R., George, A., Black, G. W. & Cummings, S. P. (2001). *Flavobacterium frigidarium* sp. nov., an aerobic, psychrophilic, xylanolytic and laminarinolytic bacterium from Antarctica. *Int J Syst Evol Microbiol* **51**, 1235–1243.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Margesin, R. & Schinner, F. (1994). Properties of cold-adapted microorganisms and their potential role in biotechnology. *J Biotechnol* **33**, 1–14.
- McCammon, S. A. & Bowman, J. P. (2000). Taxonomy of Antarctic *Flavobacterium* species: description of *Flavobacterium gillisiae* sp. nov., *Flavobacterium tegetincola* sp. nov. and *Flavobacterium xanthum* sp. nov., nom. rev. and reclassification of [*Flavobacterium*] *salegens* as *Salegentibacter salegens* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 1055–1063.
- McCammon, S. A., Innes, B. H., Bowman, J. P., Franzmann, P. D., Dobson, S. J., Holloway, P. E., Skerratt, J. H., Nichols, P. D. & Rankin, L. M. (1998). *Flavobacterium hibernum* sp. nov., a lactose-utilizing bacterium from a freshwater Antarctic lake. *Int J Syst Bacteriol* **48**, 1405–1412.
- Morita, R. Y. (1975). Psychrophilic bacteria. *Bacteriol Rev* **39**, 144–167.
- Russell, N. J. & Hamamoto, T. (1998). Psychrophiles. In *Extremophiles: Microbial Life in Extreme Environments*, pp. 25–45. Edited by K. Horikoshi & W. D. Grant. New York: Wiley.
- Sambrook, J., Frisch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Skerman, V. B. D. (1967). *A Guide to the Identification of Genera of Bacteria*, 3rd edn. Baltimore: Williams & Wilkins.
- Sly, L. I., Blackall, L. L., Kraat, P. C., Tian-Shen, T. & Sangkhobol, V. (1986). The use of second derivative plots for the determination of mol% guanine plus cytosine of DNA by the thermal denaturation method. *J Microbiol Methods* **5**, 139–156.
- Smibert, R. M. & Krieg, N. R. (1981). Phenotypic characterization. In *Manual of Methods for General Microbiology*, pp. 611–654. Edited by P. Gerhardt, R. G. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E., Koch, C., Gvozdiak, O. & Schumann, P. (1995). Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. *Int J Syst Bacteriol* **45**, 682–692.
- 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 Acid Res* **22**, 4673–4680.
- Tindall, B. J., Ross, H. N. M. & Grant, W. D. (1984). *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaeobacteria. *Syst Appl Microbiol* **5**, 41–57.
- Van de Peer, Y. & De Wachter, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.