Pedobacter cryoconitis sp. nov., a facultative psychrophile from alpine glacier cryoconite

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On the basis of phenotypic and genotypic characteristics, a novel species belonging to the genus *Pedobacter* is described. A facultatively psychrophilic, Gram-negative, aerobic, rod-shaped strain, A37^T, was isolated from alpine glacier cryoconite. The non-flagellated and non-spore-forming isolate grew over a temperature range of 1–25 °C, showed activities of oxidase, catalase, DNase, protease (gelatin, casein), amylase, β -glucosidase, β -galactosidase and β -lactamase and degraded oil hydrocarbons. A distinct optimum temperature of 15 °C was observed for both protease production and oil hydrocarbon biodegradation. Analysis of 16S rDNA revealed that strain A37^T represents a distinct taxon within *Pedobacter*. DNA from strain A37^T showed only 19.7% genetic relatedness to the DNA of *Pedobacter piscium*. The DNA G+C content was 43.4 mol%. Dominant fatty acids (51%) were iso-15:0 2-OH and 16:1 ω 7c. The strain is assigned to a novel *Pedobacter* species, for which the name *Pedobacter cryoconitis* sp. nov. is proposed, with A37^T (=DSM 14825^T=LMG 21415^T) as the type strain.

Cold-adapted micro-organisms, able to proliferate in cold environments, and their enzymes provide a large biotechnological potential, offering numerous economic and ecological advantages (Gerday *et al.*, 2000; Margesin *et al.* 2002a). A wide range of metabolic activities has been detected at low temperatures, including degradation of natural and xenobiotic organic compounds such as protein, cellulose, carbohydrates, lignin and aliphatic and aromatic hydrocarbons (Morita *et al.*, 1997; Huston *et al.*, 2000; Margesin *et al.*, 2002b). Micro-organisms able to degrade oil hydrocarbons at low temperatures are useful for the bioremediation of contaminated cold environments (Bej *et al.*, 2000; Margesin & Schinner, 2001; Yumoto *et al.*, 2002).

Adaptation strategies with regard to growth, enzyme production and enzyme activity enable cold-adapted micro-organisms to compensate for the negative effects of low temperatures on biochemical reactions. These organisms can be isolated from permanently cold environments such as fresh and marine waters, snow, glacier and sea ice, polar and high-alpine soils, cold deserts and permafrost soils. An ideal habitat is cryoconite on glacier ice (Takeuchi *et al.*, 2001; Margesin *et al.*, 2002b), a dark mixture on the surface and in microcaverns of glaciers, consisting predominantly of

organic debris (micro-organisms, pollen, plant and animal litter) and inorganic dust. Little is known of the microbial life in alpine cryoconite.

We isolated a facultatively psychrophilic representative of the genus *Pedobacter* from alpine glacier cryoconite and report the results of a phenotypic, genetic and phylogenetic examination. The strain degrades diesel oil and produces high yields of protease at low temperatures. The data obtained suggest that the isolate represents a novel species of the genus *Pedobacter*, and the name *Pedobacter cryoconitis* sp. nov. is proposed.

Cryoconite samples were collected in a ski area on the Stubai Glacier in the Tyrolean Alps (Austria) at an altitude of 3000 m above sea level and microbial strains were isolated as described by Margesin *et al.* (2002b). Morphological and phenotypic properties of the strain were investigated by using standard procedures (Süßmuth *et al.*, 1987). Amino-peptidase activity (Bactident; Merck) was tested to confirm classical Gram-staining. The biochemical profile was determined with API 20NE strips (bioMérieux) incubated at 10 and 20 °C for up to 4 days. Carbon assimilation was tested by using API 50CH strips (bioMérieux) incubated at 20 °C for up to 5 days. Sensitivity to antibiotics was determined with ATB G– and ATB G+ strips (bioMérieux) at 10 and 20 °C. Amylase, protease, lipase and β -lactamase activities were tested on nutrient broth agar plates supplemented with starch or skimmed milk (2 g each compound l^{–1}), tributyrin (5 ml l^{–1}) or ampicillin (50 mg l^{–1}). After 24–48 h at

Published online ahead of print on 21 February 2003 as DOI 10.1099/ijs.0.02436-0.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain A37^T is AJ438170.

Table 1. Phenotypic features that distinguish *Pedobacter cryoconitis* sp. nov. from other *Pedobacter* species

Data were taken from Steyn *et al.* (1998) and this study. +, Positive; –, negative; (+); weak reaction; V, variable; ND, no data available. All strains are positive for aerobic growth, production of a yellow pigment on nutrient agar, presence of catalase, oxidase and β -galactosidase and assimilation of D-glucose, D-mannose, lactose and melezitose. All strains are negative for Gram stain, motility (non-flagellated), sporulation, indole production, H₂S production from thiosulfate, nitrate reduction, presence of urease, arginine dihydrolase and tryptophan deaminase, assimilation of erythritol, L-xylose, methyl β -xyloside, L-sorbose, dulcitol, inositol, xylitol, D-tagatose, D-fucose, L-arabitol, gluconate, caprate, adipate, phenylacetate, D-lyxose, D-arabitol, 5-ketogluconate, malate and citrate and growth on MacConkey agar. All strains are susceptible to sulfamethoxazole (16 μ g) and resistant to amikacin (16 μ g).

Characteristic	<i>P. cryoconitis</i> sp. nov.	<i>P. heparinus</i>	<i>P. africanus</i>	<i>P. piscium</i>	<i>P. saltans</i>
Habitat	Glacier cryoconite	Soil	Soil, activated sludge	Fish	Soil
Growth range (°C)	1–25	5–30	ND	5–30	ND
DNA G + C content (mol%)	43.4	42–43	43–45	40–43	36–38
Assimilation of (API 50CH):					
Glycerol	–	–	–	–	+
D-Arabinose	–	–	V	–	V
L-Arabinose	+	+	V	+	+
Ribose	–	–	V	–	–
D-Xylose	+	+	V	+	+
Adonitol	–	+	–	–	+
D-Galactose	+	+	+	V	+
D-Fructose	+	+	V	+	V
Rhamnose	–	+	+	V	+
Mannitol	–	+	–	–	–
Sorbitol	–	+	–	–	–
Methyl α -D-mannoside	–	+	+	–	–
Methyl D-glucoside	(+)	+	+	+	V
Arbutin	+	V	V	+	+
Salicin	+	+	V	+	+
D-Cellobiose	+	+	+	+	–
Maltose	+	+	V	+	+
Melibiose	+	+	+	+	V
Sucrose	+	+	+	+	V
Trehalose	+	+	+	+	V
Inulin	(+)	–	–	–	–
D-Raffinose	+	–	–	+	V
Starch	+	–	V	+	–
Glycogen	+	–	–	–	–
β -Gentiobiose	+	+	V	+	+
D-Turanose	+	+	+	+	V
L-Fucose	–	+	V	–	–
2-Ketogluconate	+	–	–	–	–
Susceptibility to antibiotics (μ g):					
Ampicillin (8)	–	–	–	ND	V
Chloramphenicol (8)	–	+	V	ND	+
Cephalothin (8)	–	+	V	ND	–
Penicillin (0.3)	–	+	V	ND	V
Tetracycline (2)	–	+	–	ND	+
Gentamicin (4)	–	–	–	ND	V
Cephmandole (16)	–	+	–	ND	V
Erythromycin (4)	–	+	–	ND	V

10 °C, a positive reaction was observed when transparent zones around the colonies were visible directly or detected after precipitation or staining of the undegraded substrate. In the case of β -lactamase activity, a positive reaction was indicated by growth in the presence of ampicillin.

Strain A37^T isolated from alpine glacier cryoconite had all of the phenotypic characteristics of the genus *Pedobacter* (Steyn *et al.*, 1998) (Table 1). The cells were Gram-negative, aerobic, rod-shaped (0.7–0.9 μ m in diameter and 1.5–3.0 μ m long), non-spore-forming, non-flagellated and motile by

gliding. On R₂A agar (composition given below), colonies were round, 2–4 mm in diameter, slightly convex, with entire margins, mucoid, motile by gliding and had a light-yellow non-fluorescent pigment. Activities of oxidase, catalase, DNase, amylase, β -glucosidase, β -galactosidase, protease (gelatin and casein) and β -lactamase were present, while activities of arginine dihydrolase, urease, esterase lipase (C₄) and tryptophan deaminase, nitrate reduction, H₂S production from thiosulfate, indole production and growth on MacConkey agar were not detected.

The strain assimilated the carbohydrates L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, D-raffinose, starch, glycogen, β -gentiobiose, D-turanose, gluconate and 2-ketogluconate. Methyl α -D-glucoside and inulin were assimilated to a lesser extent. Assimilation of amygdalin and N-acetylglucosamine was only noted after 8 days of incubation. The following carbohydrates were not assimilated: glycerol, erythritol, D-arabinose, ribose, L-xylose, adonitol, methyl β -xyloside, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, 5-ketogluconate, phenylacetate, citrate, malate, adipate and caprate. Thus, strain A37^T can be distinguished from three *Pedobacter* species by its inability to assimilate rhamnose and from four *Pedobacter* species by its ability to assimilate glycogen, 2-ketogluconate and, to a lesser extent, inulin (Table 1).

The study of antibiotic susceptibility showed that strain A37^T was sensitive to sulfamethoxazole, which is a characteristic feature of *Pedobacter* species, and also to doxycycline and clindamycin. Resistance was observed to a wide range of antibiotics: penicillins (penicillin, ampicillin, mezlocillin, oxacillin, amoxicillin), cephalosporins (cephalothin, cephotaxim, cephazolin, ceftazidim, cephmandole), other β -lactams (aztreonam, imipenem), aminoglycosides (gentamicin, streptomycin), erythromycin, amikacin, chloramphenicol, tetracycline, fusidic acid and quinolones (ciprofloxacin, norfloxacin).

Analysis of fatty acid methyl esters was performed by GLC as described previously (Miller, 1982; Sasser, 1990). Dominant fatty acids (51 %) were iso-15:0 2-OH and 16:1 ω 7c. The presence of significant amounts of these two fatty acids is characteristic of all representatives of the family *Sphingobacteriaceae* (Steyn *et al.*, 1998).

Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA, purification of PCR products and electrophoresis of sequence reactions were done as described previously (Rainey *et al.*, 1996). The 16S rDNA sequence was aligned manually with published sequences from representatives of the *Cytophaga–Flavobacterium–Bacteroides* (CFB) group contained in the DSMZ database of 16S rDNA sequences. The ae2 editor (Maidak *et al.*, 1999) was used to align the 16S rDNA sequence of strain A37^T against sequences of *Pedobacter* type strains available from public

databases. Pairwise evolutionary distances were computed using the correction of Jukes & Cantor (1969). The least-squares distance method of De Soete (1983) was used in the construction of the phylogenetic dendrogram from distance matrices. Bootstrap analyses were done as described by Felsenstein (1993).

The almost-complete 16S rDNA sequence of strain A37^T, consisting of 1495 nt, was compared with sequences of members of the CFB group. Members of the genus *Pedobacter* were the closest phylogenetic neighbours. Binary similarity values ranged between 97.9 % (*Pedobacter piscium* DSM 11725^T) and 90.3 % (*Pedobacter saltans* DSM 12145^T). Similarly high values separated the type strains of *Pedobacter heparinus* (DSM 2366^T) and *Pedobacter africanus* (DSM 12126^T) (97.9 %). Distance matrix analyses placed strain A37^T in a separate line of descent, showing no close relatedness to any of the type strains of *Pedobacter* (Fig. 1).

For DNA–DNA hybridization experiments, *P. piscium* DSM 11725^T (showing >97 % 16S rDNA sequence similarity) was used as a reference strain. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite according to the procedure of Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), with the modification described by Huß *et al.* (1983) and Escara & Hutton (1980), using a model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992). DNA from strain A37^T showed only 19.7 % genetic relatedness to the DNA of *P. piscium*. Based on generally accepted criteria of the definition of genomic species (Wayne *et al.*, 1987), strain A37^T is assigned to a separate species.

The DNA G + C content for the genus *Pedobacter* ranges broadly from 36.9 mol% (reported for *P. saltans*) to 44.1 mol% (reported for *P. africanus*) (Steyn *et al.*, 1998). The G + C content of the DNA of strain A37^T was

Fig. 1. Phylogenetic dendrogram obtained by distance-matrix analysis of 16S rDNA sequences showing the position of *Pedobacter cryoconitis* sp. nov. DSM 14825^T among members of the genus *Pedobacter*. The sequence of *Flavobacterium johnsoniae* served as an outside reference. Bootstrap values >95 % are indicated at branch-points. Bar, 5 substitutions per 100 nucleotides.

43.4 mol%, determined after degradation of the DNA as described by Mesbah *et al.* (1989) and separation of the resulting nucleosides by HPLC as described by Tamaoka & Komagata (1984).

Strain A37^T was routinely cultivated in the low-strength medium R₂A, containing 0.05 % yeast extract, 0.05 % peptone, 0.05 % Casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K₂HPO₄ and 0.005 % MgSO₄ (pH 7). This medium is suitable for the cultivation of micro-organisms from oligotrophic environmental habitats. Cultivation was done aerobically at 180 r.p.m. in 100-ml Erlenmeyer flasks containing 10 ml R₂A medium with an initial cell density of 10⁸ c.f.u. ml⁻¹ and three replicates per treatment, unless indicated otherwise.

The pH tolerance (pH 5–10) for growth was determined after 48 h at 20 °C. The pH range for growth was 5–8, with optimum growth at pH 6–7.

The temperature tolerance for growth was examined at 1–30 °C in the absence and presence of oil hydrocarbons [2 g diesel oil l⁻¹ (85.7 % C, density 820 g l⁻¹)]. Cell density in cultures was determined from cell protein concentrations. Protein was measured according to Bradford (1976) after disintegration of cells by boiling in 0.1 M NaOH for 10 min (Chen & Skidmore, 1987). There was no interference of diesel oil with this method. Optical density measurements were not possible in the presence of hydrocarbons due to the formation of cell aggregates. The bacterium exhibited the properties of a facultative psychrophile (Morita, 1975), showing a growth temperature range of 1–25 °C and an optimum growth temperature of 20 °C after 24 h cultivation. After 48 h, comparable cell densities were detected over a cultivation temperature range of 10–25 °C. No growth occurred at 28 °C. Comparable cell densities were measured in cultures with and without diesel oil at all temperatures tested. Since the growth temperature range for strains of the genus *Pedobacter* is normally between 5 and 30 °C, and some strains of this genus may even grow at 37 °C (Steyn *et al.*, 1998), strain A37^T can be easily distinguished from other *Pedobacter* species by its inability to grow at 28 °C and its ability to grow well at 1 °C.

Another characteristic feature of strain A37^T is the ability to degrade oil hydrocarbons, which has not yet been described for other *Pedobacter* species. The effect of temperature on hydrocarbon biodegradation was evaluated at 1–25 °C in the dark in R₂A medium containing 2 g diesel oil l⁻¹. After 1–8 days, the residual hydrocarbon content was measured in inoculated and in sterile flasks as described previously (Margesin & Schinner, 1997). Co-metabolic oil hydrocarbon biodegradation was calculated from the difference between hydrocarbon losses in the inoculated and sterile medium. Maximum biodegradation was observed at 15 °C; cultivation at this temperature resulted in decontamination of 53 % of the diesel oil added (2 g l⁻¹) after 4 days. At lower temperatures, biodegradation was still remarkable (38–40 % at 5–10 °C, 26 % at 1 °C). After 8 days, comparable

biodegradation was observed over a temperature range of 1–15 °C. Biodegradation was negligible at 20 and 25 °C.

The effect of hydrocarbon concentration on biodegradation was examined in the same medium supplemented with 1–20 g diesel oil l⁻¹ and incubated at 15 °C for 4 days, using sterile controls for each concentration tested. Strain A37^T could grow in the presence of diesel oil concentrations up to 20 g l⁻¹; approximately 60 % of initial concentrations in the range of 1–10 g l⁻¹ was degraded after 4 days at 15 °C.

The temperature tolerance for protease formation by strain A37^T was examined in nutrient broth supplemented with skimmed milk (2 g l⁻¹) at 180 r.p.m. and 1–25 °C. Proteolytic activity was measured by digestion of azocasein, as described by Margesin & Schinner (1992a). Protease production was highest at a cultivation temperature of 15 °C, was approximately 50 % lower at 10 and 20 °C and was negligible at 1 and 25 °C. Strain A37^T also hydrolyses gelatin, a property that has been described for only one of six strains of *P. africanus* and not for *P. heparinus*, *P. piscium* or *P. saltans* (Steyn *et al.*, 1998).

Among cold-adapted micro-organisms, obligate psychrophiles are distinguished from facultative psychrophiles because of differences in ecological distribution and biochemical adaptations (Russell, 1990). Even in permanently cold habitats, such as glacier cryoconite, at least 50 % of the bacteria (Delille & Perret, 1989) or even a greater portion (Herbert, 1986) is not obligately but facultatively psychrophilic, i.e. able to grow from 0 °C to temperatures above 20 °C, with an optimum growth temperature above 15 °C. Strain A37^T belongs to this latter group. Like many cold-adapted micro-organisms, strain A37^T produces mucous polysaccharide capsules to protect the cells from environmental influences and to retain nutrients (Gounot, 1999). Although the strain was isolated from a non-contaminated environment, a remarkable oil hydrocarbon biodegradation activity was shown. This confirms that cold-adapted oil degraders are ubiquitous (Margesin & Schinner, 2001).

Temperature had a significant effect on hydrocarbon biodegradation, protease production and growth of strain A37^T. Despite high cell densities over a temperature range of 10–25 °C, a distinct optimum temperature of 15 °C was noticed for both protease production and oil hydrocarbon biodegradation. The optimum temperature for substrate utilization by cold-adapted micro-organisms is usually significantly below the optimal growth temperature of the producing strains. Thermal inhibition of extracellular enzyme production (Margesin & Schinner, 1992b) and, as demonstrated in this study, of hydrocarbon biodegradation is a common feature of these micro-organisms. The capacity of the isolate described to utilize both natural substrates (protein) and oil hydrocarbons at low temperature points to the ecological significance and biotechnological potential of the microbial community living in cryoconite.

In conclusion, the novel strain, A37^T, can be distinguished

easily from other *Pedobacter* species by its facultatively psychrophilic nature, i.e. inability to grow at 28 °C and ability to grow at 1 °C. Another characteristic feature of strain A37^T is the ability to degrade oil hydrocarbons, which has not yet been described for other *Pedobacter* species. Strain A37^T can also be distinguished from other *Pedobacter* species by its ability to assimilate glycogen and 2-ketogluconate.

Description of *Pedobacter cryoconitis* sp. nov.

Pedobacter cryoconitis (cry.o.co.ni'tis. N.L. gen. n. *cryoconitis* from cryoconite).

On nutrient agar, colonies are light-yellow, round, convex, mucoid, with entire margins. Cells are rod-shaped (0.7–0.9 µm in diameter and 1.5–3.0 µm long), Gram-negative, non-spore-forming, non-flagellated and motile by gliding. Oxidase, catalase, DNase, amylase, β-glucosidase, β-galactosidase, protease and β-lactamase are positive. Negative for activity of arginine dihydrolase, urease, lipase and tryptophan deaminase, nitrate reduction, H₂S production from thiosulfate, indole production and growth on MacConkey agar. Growth occurs between 1 and 25 °C and pH 5–8 in R₂A medium, with optimum growth at 20 °C (facultatively psychrophilic) and pH 7. No growth at 28 °C or higher. The optimum temperature for biodegradation of diesel oil and for protease production is 15 °C. The strain assimilates L-arabinose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucoside, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, D-raffinose, starch, glycogen, β-gentiobiose, D-turanose, gluconate and 2-ketogluconate. Dominant fatty acids (51 %) are iso-15:0 2-OH and 16:1ω7c. The G+C content of the DNA of the type strain is 43.4 mol%.

The type strain, A37^T (=DSM 14825^T=LMG 21415^T), was isolated from alpine glacier cryoconite on the Stubaier Glacier in the Tyrolean Alps, Austria.

References

- Bej, A. K., Saul, D. & Aislabie, J. (2000). Cold-tolerant alkane-degrading *Rhodococcus* species from Antarctica. *Polar Biol* **23**, 100–105.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chen, C.-Y. & Skidmore, D. R. (1987). Langmuir adsorption isotherm for *Sulfolobus acidocaldarius* on coal particles. *Biotechnol Lett* **9**, 191–194.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Delille, D. & Perret, E. (1989). Influence of temperature on the growth potential of southern polar marine bacteria. *Microb Ecol* **18**, 117–123.
- De Soete, G. (1983). A least squares algorithm for fitting additive trees to proximity data. *Psychometrika* **48**, 621–626.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.
- Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.
- Gerday, C., Aittaleb, M., Bentahir, M. & 11 other authors (2000). Cold-adapted enzymes: from fundamentals to biotechnology. *Trends Biotechnol* **18**, 103–107.
- Gounot, A. M. (1999). Microbial life in permanently cold soils. In *Cold-Adapted Organisms*, pp. 3–15. Edited by R. Margesin & F. Schinner. Berlin: Springer.
- Herbert, R. A. (1986). The ecology and physiology of psychrophilic microorganisms. In *Microbes in Extreme Environments*, pp. 1–23. Edited by R. A. Herbert & G. A. Codd. London: Academic Press.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Huston, A. L., Krieger-Brockett, B. B. & Deming, J. W. (2000). Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. *Environ Microbiol* **2**, 383–388.
- Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from GILFORD System 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Maidak, B. L., Cole, J. R., Parker, C. T., Jr & 11 other authors (1999). A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Res* **27**, 171–173.
- Margesin, R. & Schinner, F. (1992a). A comparison of extracellular proteases from three psychrotrophic strains of *Pseudomonas fluorescens*. *J Gen Appl Microbiol* **38**, 209–225.
- Margesin, R. & Schinner, F. (1992b). Extracellular protease production by psychrotrophic bacteria from glaciers. *Int Biodeterior Biodegrad* **29**, 177–189.
- Margesin, R. & Schinner, F. (1997). Effect of temperature on oil degradation by a psychrotrophic yeast in liquid culture and in soil. *FEMS Microbiol Ecol* **24**, 243–249.
- Margesin, R. & Schinner, F. (2001). Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl Microbiol Biotechnol* **56**, 650–663.
- Margesin, R., Feller, G., Gerday, C. & Russell, N. J. (2002a). Cold-adapted microorganisms: adaptation strategies and biotechnological potential. In *The Encyclopedia of Environmental Microbiology*, vol. 2, pp. 871–885. Edited by G. Bitton. New York: Wiley.
- Margesin, R., Zacke, G. & Schinner, F. (2002b). Characterization of heterotrophic microorganisms in alpine glacier cryoconite. *Arct Antarct Alpine Res* **34**, 88–93.
- 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.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Morita, R. Y. (1975). Psychrophilic bacteria. *Bacteriol Rev* **39**, 144–167.

- Morita, Y., Nakamura, T., Hasan, Q., Murakami, Y., Yokoyama, K. & Tamiya, E. (1997). Cold-active enzymes from cold-adapted bacteria. *J Am Oil Chem Soc* **74**, 441–444.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Russell, N. J. (1990). Cold adaptation of microorganisms. *Philos Trans R Soc London B Biol Sci* **326**, 595–608.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newslett* **20**, 1–6.
- Steyn, P. L., Segers, P., Vancanneyt, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998). Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. *Int J Syst Bacteriol* **48**, 165–177.
- Süßmuth, R., Eberspächer, J., Haag, R. & Springer, W. (1987). *Biochemisch-mikrobiologisches Praktikum*. Stuttgart: Thieme.
- Takeuchi, N., Kohshima, S. & Seko, K. (2001). Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. *Arct Antarct Alpine Res* **33**, 115–122.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yumoto, I., Nakamura, A., Iwata, H., Kojima, K., Kusumoto, K., Nodasaka, Y. & Matsuyama, H. (2002). *Dietzia psychrocaliphila* sp. nov., a novel, facultatively psychrophilic alkaliphile that grows on hydrocarbons. *Int J Syst Evol Microbiol* **52**, 85–90.