Arthrobacter psychrophenicus sp. nov., isolated from an alpine ice cave

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On the basis of phenotypic, genotypic and chemotaxonomic characteristics, a novel species belonging to the genus *Arthrobacter* is described. A facultatively psychrophilic bacterium, strain AG31^T, was isolated from an alpine ice cave. The aerobic, Gram-positive, non-spore-forming, non-motile strain exhibited a rod-coccus growth cycle and produced a yellow pigment. Good growth and phenol biodegradation occurred at a temperature range of 1–25 °C. Up to 10 mM phenol was utilized as a sole carbon source. Glucose was not assimilated. Analysis of the 16S rRNA gene revealed that strain AG31^T represents a distinct lineage within the genus *Arthrobacter*, being most closely related to *Arthrobacter sulfureus*. The level of DNA–DNA relatedness to the type strain of *A. sulfureus* was 29.9%. Anteiso-C_{15:0} was the predominant fatty acid (72%). Strain AG31^T exhibited A4 α -L-Lys-L-Glu-type peptidoglycan and contained glucose as the only cell-wall sugar. MK-10 was the predominant menaquinone, and the polar lipid pattern consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. Strain AG31^T (=DSM 15454^T=LMG 21914^T) is assigned as the type strain of a novel *Arthrobacter* species, *Arthrobacter psychrophenicus* sp. nov.

In recent years, there has been a great increase in interest in psychrophilic bacteria, because of the realization that they hold great biotechnological potential, offering numerous economical and ecological advantages (Russell, 1998; Margesin & Schinner, 1999; Margesin *et al.*, 2002). A wide diversity of cold-adapted micro-organisms can be found in various cold environments. A particular cold environment is found in caves situated in cold areas. Caves in mountain areas have a constant low temperature near 0 °C and lack freeze–thaw cycles; this is favourable to the selection of cold-adapted bacteria (Gounot, 1999). Bacteria with coryneform morphology, such as *Arthrobacter*, are widespread among bacteria found in caves (Khizhnyak *et al.*, 2003). Psychrophilic *Arthrobacter* strains were reported to be the most abundant and active bacteria in subterranean cave silts (Gounot, 1967) and were also isolated from glacier silts (Moiroud & Gounot, 1969), Arctic soils (Juck *et al.*, 2000) and Antarctic environments (Loveland-Curtze *et al.*, 1999; Reddy *et al.*, 2000, 2002; Stibor *et al.*, 2003).

Representatives of cold-adapted *Arthrobacter* strains produce cold-active enzymes with interesting properties (Nakagawa *et al.*, 2003; Stibor *et al.*, 2003). However, there is little information on *Arthrobacter* strains able to degrade hydrocarbons at low temperatures, although high-G + C coryneform bacteria are considered important members of hydrocarbon-degrading microbial populations (Bej *et al.*, 2000; Juck *et al.*, 2000; Margesin *et al.*, 2003a). A cold-adapted representative of the genus *Arthrobacter* isolated from an alpine ice cave has been described recently as degrading high phenol concentrations at low temperatures (Margesin *et al.*, 2003b). This strain was subjected to phenotypic, chemotaxonomic, genetic and phylogenetic examinations.

Strain AG31^T was isolated from a carbonate-rich deposit collected under sterile conditions in the autumn of 1999 in an alpine ice cave (Eisriesenwelt Werfen) located in Salzburg, Austria, at about 1640 m above sea level. A 10 g sample was shaken with 90 ml sterile 1% (w/v) sodium pyrophosphate for 20 min at 350 r.p.m. Appropriate dilutions, prepared with sterile distilled water, were plated (0.1 ml) on soil-extract agar incubated at 4 °C. The medium contained 50% (v/v) soil extract (pH 7), 0.05%

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(w/v) yeast extract, 0.05 % (w/v) peptone from soymeal and 1.2 % (w/v) agar (pH 7).

Genomic DNA extraction and PCR-mediated amplification of the 16S rRNA gene were done as described previously (Rainey *et al.*, 1996). PCR products were sequenced according to the manufacturer's instructions using a CEQ DCTS quick start kit (Beckman Coulter) and a CEQ8000 automatic sequencing system (Beckman Coulter). The 16S rRNA gene sequence was aligned manually with published sequences from representatives of the Actinobacteria contained in the database of 16S rRNA gene sequences held by the DSMZ. The ae2 editor (Maidak *et al.*, 1999) was used to align the 16S rRNA gene sequence of strain AG31^T against the sequences of the *Arthrobacter* 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 phylogenetic analysis included all reported species of the genus *Arthrobacter*. The analysis based on the almost complete 16S rRNA gene sequence (1501 nt) of strain AG31^T indicated that *Arthrobacter sulfureus* DSM 20167^T was the closest phylogenetic neighbour, displaying 98.3 % sequence similarity to strain AG31^T (Fig. 1).

A. sulfureus DSM 20167^T was used as reference strain for DNA–DNA hybridization. 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 modifications described by Huß *et al.* (1983) and Escara & Hutton (1980), using a model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and

plotter (Gilford Instrument Laboratories). Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992). DNA from strain AG31^T showed only 29.9 % DNA–DNA relatedness to *A. sulfureus*.

Analysis of fatty acid methyl esters was performed by GLC as described by Miller (1982) and Sasser (1990). The cellular fatty acids of strain AG31^T grown at 25 °C were identified as anteiso-C_{15:0} (72.1 %), anteiso-C_{17:0} (7.0 %), iso-C_{16:0} (8.3 %), iso-C_{15:0} (5.6 %), C_{16:0} (2.8 %), iso-C_{14:0} (2.1 %), anteiso-C_{13:0} (0.9 %), iso-C_{17:0} (0.7 %) and C_{14:0} (0.7 %). The presence of significant amounts of the predominant fatty acid anteiso-C_{15:0} is characteristic of representatives of the genus *Arthrobacter* (Keddie *et al.*, 1986); anteiso 12-methyl tetradecanoic acid is also the predominant fatty acid in *A. sulfureus* (Stackebrandt *et al.*, 1983).

The peptidoglycan structure, menaquinones and polar lipids were determined as described by Groth *et al.* (1997) and cell-wall sugars were analysed according to Stanek & Roberts (1974). Analysis of the peptidoglycan structure of strain AG31^T revealed it to be of the A4 α type (Schleifer & Kandler, 1972) with an interpeptide bridge consisting of L-Glu, type A11.54, according to the DSMZ (2001). To date, this peptidoglycan type has been reported within the genus *Arthrobacter* only for *A. sulfureus* (Schleifer & Kandler, 1972). Strain AG31^T contains menaquinones MK-10, MK-9 and MK-11 (ratio of peak areas, 72:12:1, respectively). *A. sulfureus*, the closest neighbour phylogenetically, differs from strain AG31^T in displaying MK-9 as the predominant component and possessing MK-10, MK-8 and MK-7 only in small amounts (Yamada *et al.*, 1976). *Arthrobacter nicotianae* is phylogenetically related to strain AG31^T but differs in its peptidoglycan structure and menaquinone composition (Table 1). The polar lipid pattern of strain AG31^T consists of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. Glucose was detected as the only cell-wall sugar (Table 1).

The morphological, physiological and biochemical properties of strain AG31^T were investigated and compared with those of *A. sulfureus* DSM 20167^T. Since strain AG31^T is related to a phylogenetic cluster that was designated the 'nicotianae group' (Stackebrandt *et al.*, 1983; Stackebrandt & Schumann, 2000), *A. nicotianae* DSM 20123^T, as an established representative of this group of highly related *Arthrobacter* species, was also chosen for comparison. Morphology and motility were examined by using phase-contrast microscopy (1000 $\times$) of cells grown on nutrient agar (pH 7) plates at 25 °C over 48 h. Testing for the Gram reaction was carried out using classical Gram-staining and was confirmed by the KOH-lysis test and the aminopeptidase activity test (Bactident; Merck) as described by Süßmuth *et al.* (1987). Sensitivity to antibiotics was determined with ATB strips (bioMérieux) incubated for up to 48 h at 25 °C. The biochemical profile was determined with API CORYNE and API 20NE strips (bioMérieux) incubated at 25 °C for up to 48 h; the profile of strain AG31^T

Fig. 1. Phylogenetic dendrogram based on 16S rRNA gene sequences showing part of the dendrogram obtained by distance-matrix analysis of all members of the genus *Arthrobacter* indicating the position of *A. psychrophenicus* DSM 15454^T. Bootstrap values higher than 50 % are indicated at the relevant branching points. The scale bar represents 5 nucleotide substitutions per 100 nucleotides.

Table 1. Differential phenotypic characteristics of AG31^T, *A. sulfureus* DSM 20167^T and *A. nicotianae* DSM 20123^T

All strains are positive for the following features: Gram stain; aerobic growth; production of a yellow, non-fluorescent pigment on nutrient agar; presence of catalase, α -glucosidase, pyrazinamidase, esterase (C4), esterase-lipase (C8) and leucine arylamidase; assimilation of maltose, gluconate, malate and citrate; tolerance of 1–3 % (w/v) NaCl. All strains are negative for the following features: motility; sporulation; indole production; H₂S production from thiosulfate; presence of oxidase, arginine dihydrolase, α -galactosidase, *N*-acetyl- β -glucosaminidase, lipase (C14), gelatin hydrolysis, trypsin, α -chymotrypsin, α -fucosidase, β -lactamase and pectate lyase; utilization of n-hexadecane or diesel oil for growth; assimilation of mannose, *N*-acetylglucosamine, caprate and adipate; fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose and glycogen; growth at pH 5 and 11. All strains are susceptible to ampicillin (8 mg l⁻¹) and cotrimoxazole (sulfamethoxazole + trimethoprim, 16 mg l⁻¹) and resistant to aztreonam (32 mg l⁻¹), ceftazidime (16 mg l⁻¹), ciprofloxacin and norfloxacin (8 mg l⁻¹). Symbols: +, positive; –, negative; (+); weak.

Characteristic	AG31 ^T	<i>A. sulfureus</i>	<i>A. nicotianae</i>
Source	Alpine ice cave	Oil brine	Tobacco plant
Colony diameter (mm) on nutrient agar after 48 h at 25 °C	1	1–3	0.2–0.5
Growth temperature range (°C) on nutrient agar	1–25	1–30	15–37
Phenol degradation	+	+	–
Enzymes			
Nitrate reduction	+	–	+
Urease	+	+	–
Alkaline phosphatase	–	–	+
Pyrrolidonyl arylamidase	–	–	+
Cystine arylamidase	–	(+)	–
Hydrolysis of skimmed milk at 10 °C	+	–	–
Hydrolysis of skimmed milk at 25 °C	+	+	+
Assimilation of:			
Glucose	–	+	+
Arabinose	–	–	+
Mannitol	+	+	–
Phenylacetate	+	–	–
Susceptibility to antibiotics (mg l ⁻¹)			
Mezlocillin (16)	+	–	–
Cefamandole (16)	+	–	–
Cefotaxime (16)	+	(+)	–
Tetracycline (4)	+	(+)	–
Amikazin (16)	+	(+)	(+)
Imipenem (16)	(+)	+	+
Peptidoglycan type	A4z L-Lys-L-Glu	A4z L-Lys-L-Glu ^{a*}	A4z L-Lys-L-Ala-L-Glu ^a
Major menaquinones	MK-10, MK-9, MK-11	MK-8, MK-9 ^b	MK-9, MK-10 ^b
Cell-wall sugars	Glucose	Galactose, glucose ^c	Galactose, glucose ^c

*Data from other studies were taken from: *a*, Schleifer & Kandler (1972); *b*, Yamada *et al.* (1976); *c*, Keddie & Cure (1978).

was also determined at 10 °C. The ability to utilize glucose was additionally tested by growing the strains in a pH-neutral minimal medium with glucose (0.2 %, w/v) as the sole carbon source. Thiosulfate formation, motility and indole formation were tested by using SIM agar (Süßmuth *et al.*, 1987). The presence of enzyme activities was determined with API ZYM strips incubated at 30 °C for 6 h. Additionally, amylase, protease, pectolytic enzymes, β -galactosidase, lipase and β -lactamase activities were tested on nutrient agar (pH 7) plates supplemented with starch, skimmed milk, polygalacturonic acid (each at 0.4 %, w/v), lactose and X-Gal (0.2 %, w/v, and 0.004 %, w/v, respectively) or ampicillin (50 μ g ml⁻¹) as described by Margesin *et al.* (2003b) and incubated at 10 and 25 °C.

The properties of strain AG31^T are indicated in the species description; the diagnostic characteristics that differentiate the strain from *A. sulfureus* and *A. nicotianae* are shown in Table 1. Colonies of strain AG31^T displayed a yellow, non-fluorescent pigment on nutrient agar; this pigment was lost when the strain was grown on minimal medium agar plates with phenol as the sole carbon source. In comparison, *Arthrobacter chlorophenicus* produced its pearl-grey pigment in the presence of a number of phenolic compounds only under growth conditions involving low oxygen availability (Westerberg *et al.*, 2000). A number of species of the genus *Arthrobacter*, such as *Arthrobacter aureus*, *Arthrobacter citreus*, *Arthrobacter ilicis*, *A. nicotianae*, *Arthrobacter protophormiae*, *A. sulfureus*, *Arthrobacter uratoxydans*,

Arthrobacter mysorens (Stackebrandt *et al.*, 1983; Keddle *et al.*, 1986) and *Arthrobacter flavus* (Reddy *et al.*, 2000) were described as producing a yellow pigment. On the other hand, fewer than 1% of 515 *Arthrobacter* strains isolated from caves were pigmented (Gounot, 1999). This might be an indication that strain AG31^T does not belong to the indigenous cave population but was probably introduced from outside, since caves visited by tourists contain a large number of introduced micro-organisms.

To determine the pH tolerance for growth, the strains were cultivated aerobically at 180 r.p.m. in 100 ml Erlenmeyer flasks containing 10 ml nutrient broth (pH 5–11). Growth at 10 and 25 °C was monitored over 24–72 h by measuring the optical density at 600 nm. The pH range for growth of strain AG31^T was 6–10, with optimum growth at pH 8–10 (corresponding to the alkaline conditions of the natural habitat). Growth of *A. sulfureus* and *A. nicotianae* was optimal at pH 7–9 and 7–8, respectively. None of the strains could grow at pH 5 or 11.

Salt tolerance was determined by monitoring growth in nutrient broth supplemented with 1–10% (w/v) NaCl. The presence of 1–3% (w/v) NaCl was not growth-inhibiting but actually growth-stimulating, while concentrations of 5 or 10% (w/v) NaCl inhibited growth significantly or completely, respectively.

The effect of temperature (1–37 °C) on growth was examined in complex medium (nutrient broth) and in minimal medium composed of 0.35% Na₂HPO₄·2H₂O, 0.2% KH₂PO₄, 0.1% (NH₄)₂SO₄, 0.005% Ca(NO₃)₂·4H₂O, 0.001% ammonium iron(III) citrate and 0.02% MgSO₄·7H₂O (pH 7) and containing 5 mM phenol as the sole carbon source. Growth was tested both in liquid culture and on agar plates. Strain AG31^T exhibited the properties of a facultative psychrophile (Morita, 1975), showing good growth at temperatures ranging from 1 to 25 °C. No growth occurred at 37 °C. After 24 h cultivation in complex medium, growth was optimal at 25 °C. After 48 and 72 h, however, comparable cell densities were obtained at 15–25 °C and 10–25 °C, respectively. The highest cell density was obtained at 1 °C, while *A. sulfureus* and *A. nicotianae* produced the largest amount of biomass at 25 °C and 20–30 °C, respectively. These data demonstrate the cold-adapted character of strain AG31^T. Slow growth at 30 °C was observed only after 4 days cultivation in liquid nutrient broth culture but was not observed in nutrient agar or in liquid or solid minimal medium with phenol.

None of the strains tested could utilize diesel oil (1000 mg l⁻¹) or n-hexadecane (700 mg l⁻¹) for growth, whereas both strain AG31^T and *A. sulfureus* degraded phenol. Strain AG31^T fully degraded 5 mM phenol within 2 days cultivation at 15–25 °C; full degradation had also occurred after 3 days at 10 °C and after 7 days at 1 °C. In comparison, *A. sulfureus* degraded phenol at temperatures below 20 °C slower than did strain AG31^T.

Phenol degradation by strain AG31^T was further investigated using fed-batch cultivation in minimal medium containing phenol as the sole carbon source, whereby the same culture was recontaminated with increasing (1–12.5 mM) phenol concentrations as soon as the phenol from the previous addition had disappeared (Margesin *et al.*, 2003a). The strain degraded up to 7.5 mM phenol within 3 days at 10 °C, while 10 mM phenol was utilized within 7 days; 12.5 mM phenol had a toxic effect. *A. sulfureus* even degraded 15 mM phenol at 25 °C, which may be attributable to the natural habitat (oil brine) of the strain. Since there was no abiotic loss of phenol in sterile controls, phenol disappearance was due to biodegradation. Strain AG31^T (Margesin *et al.*, 2003b, 2004) and *A. sulfureus* produced a significantly higher amount of catechol-1,2-dioxygenase than of catechol-2,3-dioxygenase, indicating the preferred oxidation of catechol by the *ortho* type of ring cleavage.

The new strain, AG31^T, can be easily distinguished from *A. sulfureus* DSM 20167^T, the strain with the closest phylogenetic relationship, by its inability to grow at 30 °C and to assimilate glucose. Inability to assimilate glucose is rarely found with *Arthrobacter* strains, since more than 90% of tested strains utilized this carbon source (Keddle *et al.*, 1986). Other characteristic properties are indicated in Table 1. Additionally, the combination of chemotaxonomic characteristics (peptidoglycan type A4α with an interpeptide bridge consisting of L-Glu; MK-10 as the predominant menaquinone) serve to distinguish the novel bacterium from all recognized *Arthrobacter* species. Therefore, on the basis of phenotypic, phylogenetic and chemotaxonomic evidence, we propose that the bacterial strain should be classified within the genus *Arthrobacter*, as the type strain of *Arthrobacter psychrophenicus* sp. nov.

Description of *Arthrobacter psychrophenicus* sp. nov.

Arthrobacter psychrophenicus (psy.chro.phe.no'li.cus. Gr. adj. *psychros* relating to cold; N.L. masc. adj. *phenolicus* relating to phenol degradation; N.L. masc. adj. *psychrophenicus* relating to phenol degradation at low temperatures).

On nutrient agar, colonies are round, convex, glossy, with entire margins and have a yellow, non-fluorescent pigment. Cells are Gram-positive, aerobic, non-spore-forming, non-motile and exhibit a rod-coccus cycle with irregular rods in the exponential growth phase and predominantly coccoid cells in the stationary growth phase. Physiological and biochemical properties are indicated in Table 1. Good growth and phenol biodegradation occur between 1 and 25 °C (facultative psychrophile). Fully degrades up to 10 mM phenol as the sole carbon source. No growth occurs at pH 5 or 11. The predominant fatty acid (72.1%) is anteiso-C_{15:0}. The peptidoglycan type is A4α L-Lys-L-Glu. MK-10 is the predominant menaquinone, while MK-9 and MK-11 occur in smaller amounts. The polar lipids are

phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid.

The type strain is AG31^T (= DSM 15454^T = LMG 21914^T), isolated from an alpine ice cave in Werfen (Salzburg), Austria.

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References

- Bej, A. K., Saul, D. & Aislabie, J. (2000). Cold-tolerant alkane-degrading *Rhodococcus* species from Antarctica. *Polar Biol* **23**, 100–105.
- 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.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- De Soete, G. (1983). A least squares algorithm for fitting additive trees to proximity data. *Psychometrika* **48**, 621–626.
- DSMZ (2001). *Catalogue of Strains*, 7th edn. Braunschweig: Deutsche Sammlung für Mikroorganismen und Zellkulturen.
- 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 (phylogeny interference package), version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.
- Gounot, A. M. (1967). Biologic role of *Arthrobacter* in subterranean soils. *Ann Inst Pasteur (Paris)* **113**, 923–945 (in French).
- 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.
- Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Demetria terrigena* gen. nov., sp. nov., a new genus of actinomycetes isolated from compost soil. *Int J Syst Bacteriol* **47**, 1129–1133.
- Huß, 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.
- 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.
- Juck, D., Charles, T., Whyte, L. G. & Greer, C. W. (2000). Polyphasic microbial community analysis of petroleum hydrocarbon-contaminated oils from two northern Canadian communities. *FEMS Microbiol Ecol* **33**, 241–249.
- 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.
- Keddie, R. M. & Cure, G. L. (1978). Cell wall composition of coryneform bacteria. In *Coryneform Bacteria*, pp. 47–83. Edited by I. J. Bousfield & A. G. Calley. London: Academic Press.
- Keddie, R. M., Collins, M. D. & Jones, D. (1986). Genus *Arthrobacter*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1288–1301. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Khizhnyak, S. V., Tausheva, I. V., Berezikova, A. A., Nesterenko, E. V. & Rogozin, D. Y. (2003). Psychrophilic and psychrotolerant heterotrophic microorganisms of middle Siberian karst cavities. *Russ J Ecol* **34**, 231–235.
- Loveland-Curtze, J., Sheridan, P. P., Gutshall, K. R. & Brenchley, J. E. (1999). Biochemical and phylogenetic analyses of psychrophilic isolates belonging to the *Arthrobacter* subgroup and description of *Arthrobacter psychrolactophilus* sp. nov. *Arch Microbiol* **171**, 355–363.
- 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. (editors) (1999). *Biotechnological Applications of Cold-Adapted Organisms*. Berlin: Springer.
- Margesin, R., Feller, G., Gerday, C. & Russell, N. J. (2002). 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., Labbé, D., Schinner, F., Greer, C. W. & Whyte, L. G. (2003a). Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. *Appl Environ Microbiol* **69**, 3085–3092.
- Margesin, R., Gander, S., Zacke, G., Gounot, A. M. & Schinner, F. (2003b). Hydrocarbon degradation and enzyme activities of cold-adapted bacteria and yeasts. *Extremophiles* **7**, 451–458.
- Margesin, R., Bergauer, P. & Gander, S. (2004). Degradation of phenol and toxicity of phenolic compounds: a comparison of cold-tolerant *Arthrobacter* sp. and mesophilic *Pseudomonas putida*. *Extremophiles* **8**, 201–207.
- Miller, L. T. (1982). A single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Moiroud, A. & Gounot, A. M. (1969). A obligatory psychrophile bacteria isolated from glacial mud. *C R Acad Sci Hebd Seances Acad Sci D* **269**, 2150–2152.
- Morita, R. Y. (1975). Psychrophilic bacteria. *Bacteriol Rev* **39**, 144–167.
- Nakagawa, T., Fujimoto, Y., Uchino, M., Takano, K. & Tomizuka, N. (2003). Isolation and characterization of psychrophiles producing cold-active β -galactosidase. *Lett Appl Microbiol* **37**, 154–157.
- 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.
- Reddy, G. S. N., Aggarwal, R. K., Matsumoto, G. I., Stackebrandt, E. & Shivaji, S. (2000). *Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica. *Int J Syst Evol Microbiol* **50**, 1553–1561.
- Reddy, G. S. N., Prakash, J. S. S., Matsumoto, G. I., Stackebrandt, E. & Shivaji, S. (2002). *Arthrobacter roseus* sp. nov., a psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. *Int J Syst Evol Microbiol* **52**, 1017–1021.
- Russell, N. J. (1998). Molecular adaptations in psychrophilic bacteria: potential for biotechnological applications. In *Advances in Biochemical Engineering/Biotechnology*, vol. 61, pp. 1–21. Edited by T. Scheper. Berlin: Springer.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC News* **20**, 1–6.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

- Stackebrandt, E. & Schumann, P. (2000).** Introduction to the taxonomy of actinobacteria. In *The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community*, 3rd edition, release 3.3. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer. <http://141.150.157.117:8080/prokPUB/index.htm>
- Stackebrandt, E., Fowler, V. J., Fiedler, F. & Seiler, H. (1983).** Taxonomic studies on *Arthrobacter nicotianae* and related taxa: description of *Arthrobacter uratoxydans* sp. nov. and *Arthrobacter sulfureus* sp. nov. and reclassification of *Brevibacterium protophormiae* as *Arthrobacter protophormiae* comb. nov. *Syst Appl Microbiol* **4**, 470–486.
- Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Stibor, M., Potocky, M., Pickova, A., Karasova, P., Russell, N. J. & Kralova, B. (2003).** Characterization of cold-active dehydrogenases for secondary alcohols and glycerol in psychrotolerant bacteria isolated from Antarctic soil. *Enzyme Microb Technol* **32**, 532–538.
- Süßmuth, R., Eberspächer, J., Haag, R. & Springer, W. (1987).** *Biochemical and Microbiological Training*. Stuttgart: Thieme (in German).
- Westerberg, K., Elväng, A. M., Stackebrandt, E. & Jansson, J. K. (2000).** *Arthrobacter chlorophenolicus* sp. nov., a new species capable of degrading high concentrations of 4-chlorophenol. *Int J Syst Evol Microbiol* **50**, 2083–2092.
- Yamada, Y., Inouye, G., Tahara, Y. & Kondo, K. (1976).** The menaquinone system in the classification of coryneform and nocardioform bacteria and related organisms. *J Gen Appl Microbiol* **22**, 203–214.