Agrococcus casei sp. nov., isolated from the surfaces of smear-ripened cheeses

Nagamani Bora,¹ Marc Vancanneyt,² Roberto Gelsomino,² Jean Swings,^{2,3} Noelle Brennan,⁴ Timothy M. Cogan,⁴ Sandra Larpin,⁵ Nathalie Desmasures,⁵ Frieda Eliskases Lechner,⁶ Reiner M. Kroppenstedt,⁷ Alan C. Ward¹ and Michael Goodfellow¹

Correspondence

Michael Goodfellow
m.goodfellow@ncl.ac.uk

¹Division of Biology, King George VIth Building, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

²BCCM/LMG Bacteria Collection, Faculteit Wetenschappen, Ghent University, B-9000 Ghent, Belgium

³Laboratorium voor Microbiologie, Faculteit Wetenschappen, Ghent University, B-9000 Ghent, Belgium

⁴Moorepark Food Research Centre, Teagasc, Fermoy, County Cork, Ireland

⁵Laboratoire de Microbiologie Alimentaire, Université de Caen Basse – Normandie, esplanade de-la-paix, 14032 Caen cedex, France

⁶Bundesanstalt für Alpenländische Milchwirtschaft, Rotholz 50a, 6200 Rotholz, Austria

⁷DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Seven Gram-positive, coryneform bacteria with virtually identical whole-organism protein patterns were isolated from the surface of smear-ripened cheeses. Representatives of these strains were the subject of a polyphasic study designed to establish their taxonomic status. The organisms formed a distinct branch in the *Microbacteriaceae* 16S rRNA gene tree and were most closely related to members of the genus *Agrococcus*, sharing sequence similarities of 95.4–98.7 %. The chemotaxonomic profiles of the strains were consistent with their classification in the genus *Agrococcus*. The combined genotypic and phenotypic data show that the isolates should be classified in the genus *Agrococcus* as representatives of a novel species. The name *Agrococcus casei* sp. nov. is proposed for this taxon. Isolate R-17892t2^T (= DSM 18061^T = LMG 22410^T) is the type strain of *Agrococcus casei* sp. nov.

The complex consortium of micro-organisms found on the surfaces of smear-ripened cheeses includes major populations of coryneform bacteria (catalase-positive irregular rods and cocci), staphylococci (catalase-positive cocci) and yeasts (Valdés-Stauber *et al.*, 1997; Carnio *et al.*, 1999; Bockelmann & Hoppe-Seyler, 2001). Until recently, cheese coryneform bacteria were assigned to groups based on a few subjectively weighted morphological and staining properties (Piton-Malleret & Gorrieri, 1992; Eliskases-Lechner & Ginzinger, 1995). Such studies have been replaced by polyphasic taxonomic investigations, which show that the coryneform component of smear-ripened cheeses contains members of novel taxa, as exemplified by the isolation and

description of *Corynebacterium casei* and *Microbacterium gubbeenense* from the surface of Gubbeen cheese (Brennan *et al.*, 2001a, b, 2002) and *Arthrobacter arilaitensis* and *Arthrobacter bergerei* from diverse French smear-ripened cheeses (Irlinger *et al.*, 2005). The present study was designed to determine the taxonomic status of a homogeneous group of coryneform bacteria isolated from smear-ripened cheeses and presumptively assigned to the genus *Agrococcus*.

The genus *Agrococcus* was proposed by Groth *et al.* (1996) for two Gram-positive, coryneform bacteria that could be distinguished from members of other genera classified in the family *Microbacteriaceae* using genotypic and phenotypic criteria. The genus currently contains four species with validly described names: *Agrococcus baldri* Zlamala *et al.* 2002; *Agrococcus citreus* Wieser *et al.* 1999; *Agrococcus jenensis* Groth *et al.* 1996; and *Agrococcus lahaulensis* Mayilraj *et al.* 2006. These taxa contain organisms isolated from compost soil and sandstone, a medieval wall painting, air and soil, respectively. Members of these species can be

Abbreviation: DAB, diaminobutyric acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Agrococcus casei* LMG 22410^T, LMG 22411, LMG 22330 and LMG 22447 are DQ168427, DQ168426, DQ168425 and DQ168424, respectively.

distinguished using a combination of phenotypic markers and together form a distinct branch in the *Microbacteriaceae* 16S rRNA gene tree (Wieser *et al.*, 1999; Lin *et al.*, 2004).

Bacteria were isolated from Gubbeen, Livarot and Tilsit cheeses following suspension and homogenization (stomacher; Interscience) of samples of the surfaces of the cheeses in sodium citrate (2 %, w/v). Tenfold dilutions were plated onto plate count agar (Biokar Diagnostic) supplemented with 3 % (w/v) sodium chloride. The isolates were grown for up to 2 days at 30 °C on tryptic soy broth (TSB; Difco) containing 1.5 % agar (Oxoid). DNA isolation and electrophoresis were done according to Gevers *et al.* (2001) and *rep*-PCR was carried out with the primer set BOXAIR according to Versalovic *et al.* (1994). Band patterns analysed using the Pearson product moment coefficient and the UPGMA clustering algorithm with Bionumerics software (Applied Maths) were compared with a database composed of type and reference strains representing species that are common on the surfaces of smear-ripened cheeses (data not shown). A homogeneous group comprising seven isolates (LMG 22410^T, LMG 22411, LMG 22330, LMG 22447, R-21294, R-17921 and R-17924) remained unidentified. The taxonomic position of these isolates was the subject of the present study.

Five strains, LMG 22410^T (=DSM 18061^T=R-17892t2^T=BG1:37:24), LMG 22411 (=R-17926=BG1:37:9), R-21294 (=BG4:L10), R-17921 (=BG1:23:67) and R-17924 (BG1:37:21), were isolated from Gubbeen cheese, one strain, LMG 22447 (=R-19148=BIV-1M43), was from Livarot cheese, and one strain, LMG 22330 (=R-18889=B-Y413), was from Tilsit cheese. Four representative strains, namely isolates LMG 22410^T, LMG 22411, LMG 22330 and LMG 22447, were selected for further study. The reference type strains used in the present study were *A. baldri* LMG 23057^T (=DSM 14215^T), *A. citreus* LMG 23056^T (=DSM 12453^T) and *A. jenensis* LMG 23058^T (=DSM 9580^T).

The phylogenetic positions of the four representative cheese isolates were determined by 16S rRNA gene sequence analysis. Biomass from growth in brain-heart infusion broth (BHI; Difco) for 5 days at 30 °C was checked for purity, harvested by centrifugation, washed in NaCl/EDTA buffer (0.1 M EDTA, 0.1 M NaCl, pH 8.0) and stored at -20 °C until required. Genomic DNA was extracted as described by Sambrook & Russell (2001) and used as a template for PCR amplification and sequencing following the procedure of Kim *et al.* (1998). The resultant almost complete 16S rRNA gene sequences (1466–1470 nt) were manually aligned with corresponding sequences of representatives of the genera classified in the family *Microbacteriaceae*, retrieved from the GenBank and RDP databases, using the pairwise alignment option and 16S rRNA secondary structure information held in the program PHYDIT (available at <http://plaza.snu.ac.kr/~jchun/phydit/>).

Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). The distance model of Jukes & Cantor (1969) was used to generate evolutionary distance matrices for the least-squares and neighbour-joining algorithms. The resultant unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset using the CONSENSE and SEQBOOT options from the PHYLIP package. Phylogenetic analyses showed that the four isolates formed a distinct lineage within the evolutionary radiation occupied by genera classified in the family *Microbacteriaceae* (Fig. 1). This taxon was most closely related to four representatives of the genus *Agrococcus*, namely *A. baldri*, *A. citreus*, *A. lahaulensis* and *A. jenensis*, with 16S rRNA gene sequence similarities of 95.4–98.7 %.

The four isolates were the subject of chemotaxonomic studies designed to determine whether they had chemical features that were consistent with their classification in the genus *Agrococcus*. The strains were grown in BHI broth for 5 days at 30 °C and the resultant biomass was washed twice in distilled water and freeze-dried. Established methods were used for the extraction and analysis of fatty acids (Komagata & Suzuki, 1987), muramic acid type (Uchida *et al.*, 1999), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Schaal, 1985). Qualitative analysis of the amino acids was performed using a standard procedure (Schleifer & Kandler, 1972; Schleifer, 1985). The peptidoglycan structures of isolates LMG 22411 and LMG 22330 were determined using the DSMZ Identification Service. To this end, quantitative analyses of amino acids of the isolates in total hydrolysates were performed by GC and GC-MS, as described by Mackenzie (1987).

The isoprenoid quinone composition of strain LMG 22410^T was determined using modifications of established procedures (Collins & Kroppenstedt, 1987; Kroppenstedt, 1985). Isoprenoid quinones were extracted from lyophilized cells (500 mg) with chloroform:methanol (2:1, v/v) for 2 h on a magnetic stirrer. The extract was filtered and the cell biomass was re-extracted for another 2 h. Extracts were combined and evaporated (Zymark evaporator) to dryness at 40 °C; the lipids were dissolved in 200 µl acetone. Isoprenoid quinones were purified by TLC on Kieselgel 60F₂₅₄ plastic-backed TLC sheets (Merck) using the solvent system hexane:diethyl ether (85:15, v/v). Isoprenoid quinones were visualized under UV light (254 nm) and eluted from the TLC plate with diethyl ether. The eluate was dried, dissolved in 200 µl 2-propanol and filtered through a 2 µm hollow fibre syringe filter (DynaGard). The purified extract (5 µl) was analysed by HPLC on a reversed-phase column (250 × 4.6 mm Lichrospher 100 RP-18 endcapped; Merck) at 40 °C, eluted with acetonitrile:2-propanol (65:35, v/v) at 1 ml min⁻¹ using an HP-1090 HPLC pump (Hewlett Packard) and an HP-1090 diode array

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing the position of representative strains of *Agrococcus casei* sp. nov. in the *Microbacteriaceae* gene tree. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. F and M indicate branches recovered using the least-squares and maximum-likelihood methods, respectively. Numbers at the nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are given. Bar, 0.1 nucleotide substitutions per nucleotide position.

detector with HP-chemstation software. Isoprenoid quinones were identified by their retention times using bacterial quinone extracts of known composition for comparison. A spectrum of 200–400 nm at the apex of the peak was determined using a diode array detector.

The four representative isolates contained diaminobutyric acid (DAB) as the wall diamino acid. In addition, alanine, glycine and glutamic acid were detected in the two isolates that were the subject of additional studies. Cell wall Ala:Gly:DAB:Glu ratios were 2.9:1.3:0.9:1.0 and 1.8:1.0:0.7:1.0 for strains LMG 22411 and LMG 22330, respectively. The partial hydrolysates of these strains included the peptides Gly–Glu and Ala–Ala, but not L-Ala–D-Glu. The qualitative amino acid composition of strains LMG 22411 and LMG 22330 corresponded to peptidoglycan type B2 γ (Schleifer & Kandler, 1972). The isolates contained *N*-acetylated muramic acid. They contained diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid and unknown polar lipids as major polar lipids and four unknown phospholipids as minor components. Whole-organism sugars galactose, glucose and mannose were present and menaquinones MK-10 (4% of total), MK-11 (71% of total) and MK-12 (20% of total) were the predominant isoprenologues. The fatty acid profiles of the strains were rich in 12-methyltetradecanoic

(anteiso-C_{15:0}; 44.9–56.1% of total), 14-methylhexadecanoic (anteiso-C_{17:0}; 26.9–30.0% of total) and 13-methyltetradecanoic (iso-C_{15:0}; 8.2–11.6% of total) acids, with smaller proportions of 14-methylpentadecanoic acid (iso-C_{16:0}; 2.9–8.0% of total) and traces of hexadecanoic acid (C_{16:0}). These chemotaxonomic characteristics are consistent with the classification of the isolates in the genus *Agrococcus* (Groth *et al.*, 1996; Wieser *et al.*, 1999; Zlamala *et al.*, 2002), but they also suggest that the isolates belong to a separate species.

The DNA G+C content was determined for strain LMG 22410^T. DNA was extracted from biomass using the protocol described by Pitcher *et al.* (1989) modified by lysing the washed cell pellet in 10 mM Tris/HCl, 100 mM EDTA, pH 8.0, containing RNase (200 μ g ml⁻¹; Sigma), mutanolysin (100 U ml⁻¹; Sigma) and lysozyme (25 mg ml⁻¹; SERVA) for 1 h at 37 °C, then adding proteinase K (200 μ g ml⁻¹; Merck) to the mixture for 15 min before addition of guanidine isothiocyanate. The DNA was digested enzymically to nucleotides (Mesbah *et al.*, 1989). The nucleotides were analysed by HPLC on a Waters SymmetryShield C8 column maintained at 37 °C with 0.02 M NH₄H₂PO₄ (pH 4.0) and 1.5% acetonitrile as eluent; non-methylated γ -phage DNA (Sigma) was used as the calibration reference. The DNA G+C content of strain

Fig. 2. SDS-PAGE patterns of strains of *Agrococcus casei* sp. nov. and the type strains of related *Agrococcus* species. The dendrogram was constructed by the UPGMA linkage of correlation coefficients (r , expressed for convenience as percentage values).

LMG 22410^T was 65 mol%, a figure considerably lower than the values of 74–75 mol% reported in the literature for the members of the genus *Agrococcus* studied here (Groth *et al.*, 1996; Wieser *et al.*, 1999; Zlamala *et al.*, 2002).

SDS-PAGE of whole-organism proteins, a procedure commonly used for species delineation, was performed as a screening procedure to establish relationships between the isolates and *A. baldri* LMG 23057^T, *A. citreus* LMG 23056^T and *A. jenensis* LMG 23058^T. Cultures were grown for 1–2 days at 30 °C on TSB containing 1.5% agar. Whole-organism protein extracts were prepared and SDS-PAGE was carried out according to Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of protein profiles and numerical analyses were achieved using the software package GELCOMP versions 3.1 and 4.0 (Applied Maths). The isolates formed a homogeneous group that was distinct from that of the *Agrococcus* type strains studied (Fig. 2).

The representative isolates and type strains of the three *Agrococcus* species studied were examined for a range of phenotypic properties using standard procedures: acid production from sugars (Hugh & Leifson, 1953); catalase activity, degradation tests and growth on organic acids as sole carbon sources (Gordon & Mihm, 1957, 1962); aesculin hydrolysis (Williams *et al.*, 1983); allantoin and urea hydrolysis (Cowan, 1974); nitrate reduction and hydrogen sulphide production (Gordon, 1968); degradation of Tweens (Sierra, 1957); and the breakdown of glycerol tributyrin using tributyrin agar (Sigma). All tests were incubated at 30 °C for 14 days. API ZYM tests (API bioMérieux) were performed following the manufacturer's instructions. The isolates shared a range of phenotypic properties that enabled them to be distinguished from the *Agrococcus* type strains studied (Table 1).

It can be concluded from the genotypic and phenotypic data that the newly isolated strains should be classified as representatives of a novel species in the genus *Agrococcus*. The name proposed for this taxon is *Agrococcus casei* sp. nov.

Description of *Agrococcus casei* sp. nov.

Agrococcus casei (ca.se'i. L. gen. n. *casei* of cheese, named because the organism was isolated from smear-ripened cheeses).

Aerobic, Gram-positive, asporogenous, non-motile, catalase-positive actinomycete that forms irregular, ovoid to short rods. Cells are 0.3–0.4 × 0.8–1.0 µm and occur singly, in pairs or short chains and in small irregular clusters. Circular, cream-coloured, smooth colonies with entire edges are formed on tryptic soy agar. Grows at 20–30 °C, but not at 4 or 42 °C. Good growth occurs between pH 6.0 and 10.0. Starch is degraded and Tween 80 is hydrolysed. Growth on a range of carbon sources is variable. Additional phenotypic properties are shown in Table 1. The cell wall diamino acid is DAB; alanine, glycine and glutamic acid are also found. The peptidoglycan type is B2γ. Whole-organism sugars are galactose, glucose and mannose. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid, unknown polar lipids and four uncharacterized phospholipids. The predominant fatty acids are 12-methyltetradecanoic, 14-methylhexadecanoic and 13-methyltetradecanoic acids. The main menaquinones are MK-11 and MK-12. Representative

Table 1. Characteristics that enable *Agrococcus casei* sp. nov. to be distinguished from the type strains of *Agrococcus* species

Taxa: 1, *A. casei* LMG 22410^T, LMG 22411, LMG 22330 and LMG 22447; 2, *A. baldri* LMG 23057^T; 3, *A. citreus* LMG 23056^T; 4, *A. jenensis* LMG 23058^T; 5, *A. lahaulensis* DSM 17612^T.

Characteristic	1	2	3	4	5*
Aesculin hydrolysis	–	+	+	+	–
Degradation of:					
Tributyrin	–	+	+	+	ND
Tween 20	–	–	–	+	ND
Tween 80	+	+	+	–	ND
Acid production from:					
Cellobiose	–	+	+	+	–
Fructose	–	–	+	+	+
Glucose	+	+	+	–	ND
Maltose	–	+	+	–	–
Mannitol	–	+	+	+	–
Mannose	+	–	–	–	ND
Sucrose	–	+	–	–	–
Trehalose	–	+	+	–	+

*Data taken from Mayilraj *et al.* (2006). ND, Not determined.

†Data not in line with Wieser *et al.* (1999).

strains were isolated from the surfaces of smear-ripened cheeses.

The type strain is R-17892t2^T (=DSM 18061^T=LMG 22410^T), isolated from the surface of Gubbeen cheese. The DNA G+C content of the type strain is 65 mol%.

Acknowledgements

This study was carried out with financial support from the European Union [project QLK-CT-2001-02208: Biodiversity and anti-listerial activity of surface microbial consortia from Limburger, Reblochon, Livarot, Tilsit and Gubbeen cheese. Acronym: SCM (Smear cheese microflora)].

References

- Bockelmann, W. & Hoppe-Seyler, T. (2001). The surface flora of bacterial smear-ripened cheeses from cows' and goats' milk. *Int Dairy J* 11, 307–314.
- Brennan, N. M., Brown, R., Goodfellow, M., Ward, A. C., Beresford, T. P., Vancanneyt, M., Cogan, T. M. & Fox, P. F. (2001a). *Microbacterium gubbeenense* sp. nov., from the surface of a smear-ripened cheese. *Int J Syst Evol Microbiol* 51, 1969–1976.
- Brennan, N. M., Brown, R., Goodfellow, M., Ward, A. C., Beresford, T. P., Simpson, P. J., Fox, P. F. & Cogan, T. M. (2001b). *Corynebacterium mooreparkense* sp. nov. and *Corynebacterium casei* sp. nov., isolated from the surface of a smear-ripened cheese. *Int J Syst Evol Microbiol* 51, 843–852.
- Brennan, N. M., Ward, A. C., Beresford, T. P., Fox, P. F., Goodfellow, M. & Cogan, T. M. (2002). Biodiversity of the bacterial flora on the surface of a smear cheese. *Appl Environ Microbiol* 68, 820–830.
- Carnio, M. C., Eppert, I. & Scherer, S. (1999). Analysis of the bacterial surface ripening flora of German and French smeared cheeses with respect to their anti-listerial potential. *Int J Food Microbiol* 47, 89–97.
- Collins, M. D. & Kroppenstedt, R. M. (1987). Structures of the partially saturated menaquinones of *Glycomyces rutgersensis*. *FEMS Microbiol Lett* 44, 215–219.
- Cowan, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. London: Cambridge University Press.
- Eliskases-Lechner, F. & Ginzing, W. (1995). The bacterial flora of surface-ripened cheese with special regard to coryneforms. *Lait* 75, 571–584.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome c sequences is of general applicability. *Science* 155, 279–284.
- Gevers, D., Huys, G. & Swings, J. (2001). Applicability of *rep*-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol Lett* 205, 31–36.
- Gordon, R. E. (1968). The taxonomy of soil bacteria. In *The Ecology of Soil Bacteria*, pp. 293–321. Edited by T. R. G. Gray & D. Parkinson. Liverpool: Liverpool University Press.
- Gordon, R. E. & Mihm, J. M. (1957). A comparative study of some strains received as nocardiae. *J Bacteriol* 73, 15–27.
- Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* 98, 628–636.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* 46, 234–239.
- Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *J Bacteriol* 66, 24–26.
- Irlinger, F., Bimet, F., Delettre, J., Lefevre, M. & Grimont, P. A. D. (2005). *Arthrobacter bergerei* sp. nov. and *Arthrobacter arilaitensis* sp. nov., novel coryneform species isolated from the surfaces of cheeses. *Int J Syst Evol Microbiol* 55, 457–462.
- 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.
- Kim, S. B., Falconer, C., Williams, E. & Goodfellow, M. (1998). *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydophilic species from soil. *Int J Syst Bacteriol* 48, 59–68.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* 18, 1–32.
- Komagata, K. & Suzuki, K. I. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–206.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–179. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Lin, Y. C., Uemori, K., De Briel, D. A., Arunpairojana, V. & Yokota, A. (2004). *Zimmermannella helvola* gen. nov., sp. nov., *Zimmermannella alba* sp. nov., *Zimmermannella bifida* sp. nov., *Zimmermannella faecalis* sp. nov. and *Leucobacter albus* sp. nov., novel members of the family Microbacteriaceae. *Int J Syst Evol Microbiol* 54, 1669–1676.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the N-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* 70, 151–160.
- Mayilraj, S., Suresh, K., Schumann, P., Kroppenstedt, R. M. & Saini, H. S. (2006). *Agrococcus lahaulensis* sp. nov., isolated from a cold desert of the Indian Himalayas. *Int J Syst Evol Microbiol* 56, 1807–1810.
- 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.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 2, 233–241.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* 8, 151–156.
- Piton-Malleret, C. & Gorrieri, M. (1992). Nature et variabilité de la flore microbienne dans la morge des fromages de Comté et de Beaufort. *Lait* 72, 143–164 (in French).
- Pot, B., Vandamme, P. & Kersters, K. (1994). Analysis of electrophoretic whole-organism protein fingerprints. In *Modern Microbial Methods (Chemical Methods in Prokaryotic Systematics Series)*, pp. 493–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.

- Sambrook, J. & Russell, D. W. (2001).** *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schaal, K. P. (1985).** Identification of clinically significant actinomycetes and related bacteria using chemical techniques. In *Chemical Methods in Bacterial Systematics*, pp. 359–381. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Schleifer, K. H. (1985).** Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972).** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Sierra, G. (1957).** A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of contact between cells and fatty substrates. *Antonie van Leeuwenhoek* **23**, 15–22.
- Uchida, K., Kudo, T., Suzuki, K. & Nakase, T. (1999).** A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.
- Valdés-Stauber, N., Scherer, S. & Seiler, H. (1997).** Identification of yeasts and coryneform bacteria from the surface microflora of brick cheeses. *Int J Food Microbiol* **34**, 115–129.
- Versalovic, J., Schneider, M., de Bruijn, F. J. & Lupski, J. R. (1994).** Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods Mol Cell Biol* **5**, 25–40.
- Wieser, M., Schumann, P., Martin, K., Altenburger, P., Burghardt, J., Lubitz, W. & Busse, H.-J. (1999).** *Agrococcus citreus* sp. nov., isolated from a medieval wall painting of the chapel of Castle Herberstein (Austria). *Int J Syst Bacteriol* **49**, 1165–1170.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983).** Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Zlamala, C., Schumann, P., Kämpfer, P., Rosselló-Mora, R., Lubitz, W. & Busse, H.-J. (2002).** *Agrococcus baldri* sp. nov., isolated from the air in the ‘Virgilkapelle’ in Vienna. *Int J Syst Evol Microbiol* **52**, 1211–1216.