Brachybacterium muris sp. nov., isolated from the liver of a laboratory mouse strain

Sandra Buczolits,^{1,4} Peter Schumann,² Gerhard Weidler,³ Christian Radax³ and Hans-Jürgen Busse^{1,4}

Correspondence

Hans-Jürgen Busse
Hans-Juergen.Busse@
vu-wien.ac.at

¹Institut für Mikrobiologie und Genetik, Universität Wien, A-1030 Wien, Austria

²DSMZ – Deutsche Sammlung für Mikroorganismen und Zellkulturen, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

³Institut für Genetik und Allgemeine Biologie, Universität Salzburg, A-5020 Salzburg, Austria

⁴Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

A coccoid- to ovoid-shaped, Gram-positive bacterial strain, designated C3H-21^T, was isolated from the liver of the laboratory mouse strain C3H/He and characterized by a polyphasic approach. The peptidoglycan type was variation A4_γ with *meso*-diaminopimelic acid as the diagnostic cell-wall diamino acid and an interpeptide bridge of D-Asp-D-Glu. The isolate contained menaquinone MK-7 (88 %) as the major component of the quinone system and minor amounts of menaquinone MK-8 (9 %) and menaquinone MK-6 (3 %). The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, unidentified glycolipids and unidentified phospholipids. The fatty acid profile contained predominantly anteiso-C_{15:0} and significant amounts of iso-C_{16:0}, iso-C_{14:0}, anteiso-C_{17:0} and C_{19:0}. The polyamine pattern consisted of spermine and spermidine as the major compounds. Genomic fingerprints clearly distinguished strain C3H-21^T from other *Brachybacterium* species. The isolate shared the highest 16S rDNA sequence similarities with members of the genus *Brachybacterium*, in particular *Brachybacterium sacelli* LMG 20345^T, *Brachybacterium nesterenkovi* DSM 9573^T, *Brachybacterium rhamnosum* LMG 19848^T, *Brachybacterium alimentarium* CNRZ 925^T and *Brachybacterium fresconis* LMG 20336^T (97.8–97.2 %). The results of biochemical/physiological characterization, chemotaxonomic characteristics and REP-PCR-generated fingerprints demonstrated that the isolate represents a novel species of the genus *Brachybacterium*, for which the name *Brachybacterium muris* (type strain C3H-21^T = DSM 14560^T = CCM 7047^T) is proposed.

In the course of a study concerning isolation of bacteria from the livers of different laboratory mice strains (C3H/He and C57Bl/6J), several bacterial strains were isolated. Based on preliminary classification, these isolates were identified as members of the genera *Brachybacterium*, *Microbacterium*, *Propionibacterium* and *Lactobacillus*, and the family *Enterobacteriaceae*. Until now, only *Helicobacter hepaticus* has been recovered from the livers of mice and its relation to hepatitis and hepatic carcinoma has been

discussed (Fox *et al.*, 1994). On the basis of serological and molecular biological methods, the presence of other bacterial taxa in the livers of mice has also been detected, including lactobacilli (de Waard *et al.*, 2003) and propionibacteria (Tsuiji *et al.*, 1997; Sakao *et al.*, 1999), but nothing is known about their pathogenicity. In the course of our study, we isolated a strain (C3H-21^T) from the liver of a laboratory mouse which was preliminarily identified as a member of the genus *Brachybacterium*. Since the liver of mice is an unusual source for isolation of *Brachybacterium*, we subjected strain C3H-21^T to a detailed taxonomic characterization. For comparison, *Brachybacterium faecium* CCM 4372^T (Collins *et al.*, 1988), *Brachybacterium conglomeratum* CCM 2589^T, *Brachybacterium rhamnosum* DSM 10240^T (Takeuchi *et al.*, 1995), *Brachybacterium alimentarium* CCM 4520^T, *Brachybacterium tyrofermentans* CCM 4521^T (Schubert *et al.*, 1996), *Brachybacterium fresconis* DSM 14564^T, *Brachybacterium sacelli* DSM 14566^T (Heyrman *et al.*, 2002) and *Brachybacterium*

Published online ahead of print on 6 June 2003 as DOI 10.1099/ijs.0.02728-0.

Abbreviation: REP-PCR, repetitive extragenic palindromic DNA-polymerase chain reaction.

The EMBL accession number for the 16S rDNA sequence of *Brachybacterium muris* C3H-21^T is AJ537574.

An image showing the REP-PCR fingerprints of *B. muris* C3H-21^T and related *Brachybacterium* spp. is available in IJSEM Online.

nesterenkovi CCM 2432, which had been reliably identified by DNA–DNA hybridizations (Takeuchi *et al.*, 1995), were also studied.

Laboratory mice were killed with chloroform and the liver was prepared under semi-sterile conditions. Liver specimens were transferred to *Brucella* broth (Difco Laboratories) supplemented with 8 % fetal bovine serum (Life Technologies) and Skirrow selective supplement (Oxoid) and incubated under microaerobic conditions (90 % N₂, 5 % CO₂, 5 % H₂ and residual O₂) at 37 °C in an anaerobic jar (Oxoid) as reported by Fox *et al.* (1994). Samples of the enrichment culture were subcultured on sheep blood agar until pure cultures were obtained (Buczolits *et al.*, 2001).

16S rDNA amplification and sequence comparisons were done as described previously (Buczolits *et al.*, 2002). The 16S rDNA sequence of strain C3H-21^T consisted of a fragment of 1429 bases (positions 7–1438, *Escherichia coli* numbering; Brosius *et al.*, 1978). Highest but almost identical sequence similarities were found to *B. sacelli* LMG 20345^T (97.8 %), *B. nesterenkovi* DSM 9573^T (97.7 %), *B. rhamnosum* LMG 19848^T (97.7 %), *B. alimentarium* CNRZ 925^T (97.2 %) and *B. fresconis* LMG 20336^T (97.2 %). Sequence similarities to other species of the genus *Brachybacterium* were in the narrow range of 96.7–96.9 %; sequence similarity to another species of the family *Dermabacteraceae*, *Dermabacter hominis* DSM 7083^T, was 95.7 %. These results clearly demonstrated that strain C3H-21^T is affiliated to the genus *Brachybacterium* and, since it exhibited almost identical sequence similarity values to three species of the genus, they suggested that it represents a novel species of the genus *Brachybacterium*. Phylogenetic analyses confirmed the view that strain C3H-21^T is affiliated to the genus *Brachybacterium* (Fig. 1), but its position within the genus is not very clear since the branching order between many species of the genus was not supported by high bootstrap values (< 70 %).

Methods for morphological (Buczolits *et al.*, 2002), physiological and biochemical characterization have been described previously (Zlamala *et al.*, 2002). Gram-staining, KOH test and aminopeptidase test were positive. No motility was observed by phase-contrast microscopy. Strain C3H-21^T grew best on sheep blood agar and *Brucella* broth supplemented with 8 % fetal bovine serum (Buczolits *et al.*, 2001) at 25–37 °C. Strain C3H-21^T grew under aerobic conditions, but better growth was observed when it was incubated in an anaerobic jar under microaerobic conditions (90 % N₂, 5 % CO₂, 5 % H₂ and residual O₂). No growth occurred under anaerobic conditions. Other characteristics of strain C3H-21^T and other representatives of the genus *Brachybacterium* are summarized in Table 1 and in the species description below. Based on its biochemical and physiological properties, strain C3H-21^T is clearly distinguishable from the next related species.

Purified cell-wall preparations were obtained by the method of Schleifer & Kandler (1972) and the amino acids and

Fig. 1. Phylogenetic relationship of *Brachybacterium muris* C3H-21^T to other currently recognized *Brachybacterium* species, based on a comparison of almost-complete 16S rRNA gene sequences (accession numbers in parentheses). Jukes–Cantor-corrected phylogenetic distances were assessed with the program DNADIST (Felsenstein, 1993) and the tree was constructed using the neighbour-joining method of Saitou & Nei (1987). *Dermabacter hominis* was used as an outgroup. Bootstrap values are indicated at nodes supported by more than 70 of 100 replicate trees. Bar, 1 % nucleotide sequence difference.

peptides in peptidoglycan hydrolysates were analysed as described previously (Groth *et al.*, 1997). The amino acids present in the peptidoglycan of strain C3H-21^T were meso-diaminopimelic acid, alanine, glycine, aspartic acid and glutamic acid (molar ratio 1.2:1.6:1.2:1.0:2.0, respectively). On the basis of the molar ratios of the amino acids, the peptidoglycan type of the strain is A31.3 (*DSMZ Catalogue of Strains*, 7th edn, 2001). The peptidoglycan type of our isolate was variation A4γ [meso-diaminopimelic acid-(D-Glu)₂ type] (Schleifer & Kandler, 1972). The interpeptide bridge of strain C3H-21^T contains D-Asp-D-Glu rather than D-Glu-D-Glu. Strain C3H-21^T shares this peptidoglycan type with *B. alimentarium* and *B. tyrofermentans* and it differentiates it from *B. nesterenkovi*, which lacks glycine and aspartic acid, and from *B. faecium*, *B. fresconis*, *B. sacelli*, *B. rhamnosum*, *B. conglomeratum* and *Brachybacterium paraconglomeratum*, which lack aspartic acid. Muramic acid residues of the peptidoglycan of strain C3H-21^T are N-acetylated and not N-glycolated like other species of the genus *Brachybacterium* (Collins *et al.*, 1988; Gvozdyak *et al.*, 1992; Takeuchi *et al.*, 1995; Schubert *et al.*, 1996; Heyrman *et al.*, 2002). These results indicate that strain C3H-21^T is not affiliated to any established species of the genus *Brachybacterium*.

The quinone systems of strain C3H-21^T and the other *Brachybacterium* strains were analysed according to Tindall (1990). Strain C3H-21^T contained the major menaquinone MK-7 and minor amounts of menaquinone MK-8 and MK-6 (Table 1). The quinone systems of strain C3H-21^T

Table 1. Characteristics useful for differentiating strain C3H-21^T from other species of the genus *Brachybacterium*

Species: 1, *B. muris* C3H-21^T; 2, *B. sacelli* DSM 14566^T; 3, *B. nesterenkovi* CCM 2432; 4, *B. rhamnosum* DSM 10240^T; 5, *B. alimentarium* CCM 4520^T; 6, *B. fresconis* DSM 14564^T; 7, *B. paraconglomeratum* DSM 46341^T; 8, *B. faecium* CCM 4372^T; 9, *B. tyrofermentans* CCM 4521^T; 10, *B. conglomeratum* CCM 2589^T. All data for established species were generated during this study but characteristics for *B. paraconglomeratum* DSM 46341^T were taken from Takeuchi *et al.* (1995). +, Positive; (+), weakly positive; –, negative; ND, not determined. MK, menaquinone. All strains contained the polar lipids diphosphatidylglycerol, phosphatidylglycerol and the two unknown glycolipids GL1 and GL2. All strains were positive in tests for catalase, decomposition of aesculin and acid production from L-arabinose, D-galactose and D-glucose, and grew well at pH 6.0–9.0, in the temperature range 15–28 °C and in the presence of 0–10% (w/v) NaCl, but not 20% (w/v) NaCl. Results for D-galactose and D-glucose were not in line with those reported for *B. faecium* NCIB 9860^T (Takeuchi *et al.*, 1995) and results for DL-lactose, maltose and sucrose were not in line with those reported for *B. sacelli* DSM 14566^T and *B. fresconis* DSM 14564^T (Heyrman *et al.*, 2002). All strains were negative in tests for oxidase and Voges–Proskauer and decomposition of tyrosine, and were non-haemolytic. Results for oxidase were not in line with those reported for *B. alimentarium* CNRZ 925^T and *B. tyrofermentans* CNRZ 926^T (Schubert *et al.*, 1996).

Characteristic	1	2	3	4	5	6	7	8	9	10
Urease production	–	–	–	+	+	+	+	–	–	+
H ₂ S production	–	–	+	+	–	–	+	–	–	+
Indole formation	–	–	–	–	–	–	+	(+)	–	(+)
Nitrate reduction	–	+	+	+	+	+	+	+	+	+
Growth at:										
4 °C	–	–	–	+	+	–	ND	+	+	–
37 °C	+	(+)	+	+	–	(+)	ND	+	–	+
42 °C	(+)	–	–	–	–	–	ND	(+)	–	(+)
Growth at:										
pH 5.0	–	+	(+)	+	(+)	+	–	–	–	–
pH 10.0	+	–	–	–	–	+	ND	–	–	+
Growth in the presence of 15% NaCl	–	+	–	–	+	+	(+)	+	(+)	+
Hydrolysis of:										
Casein	–	–	–	+	+	–	ND	–	–	–
Starch	+	–	–	+	+	–	+	+	–	+
Tween 20	+	–	+	–	+	–	–	–	–	–
Tween 80	(+)	–	–	–	–	–	–	–	–	–
Acid production from:										
D-Arabinose	+	–	–	–	(+)	–	–	(+)	–	–
D-Fructose	+	+	–	+	–	+	+	–	–	–
DL-Lactose	+	–	+	–	–	–	(+)	–	–	–
Maltose	+	–	(+)	+	–	–	+	(+)	–	+
D-Mannose	(+)	(+)	–	+	(+)	+	+	–	+	–
Melibiose	–	–	–	+	+	+	ND	+	+	+
Raffinose	–	–	(+)	+	+	+	–	–	(+)	–
L-Rhamnose	+	(+)	–	–	+	(+)	–	–	(+)	–
D-Ribose	+	–	(+)	(+)	(+)	–	–	(+)	(+)	–
D-Sorbitol	–	–	–	+	–	–	–	–	–	–
Sucrose	+	–	+	+	–	–	+	–	–	–
D-Xylose	–	–	+	(+)	(+)	(+)	–	–	–	–
Peptidoglycan with:										
Glycine	+	+	–	+	+	+	+	+	+	+
Aspartic acid	+	–	–	–	+	–	–	–	+	–
Distinguishing unknown polar lipid(s):										
Glycolipid	GL3	GL3		GL3		GL3	GL3	GL3	GL3	GL3
Phospholipid	PL1, 2	PL1, 2	PL1–3	PL1–3	PL1	PL1, 2		PL1–4	PL1, 2	PL1–3
Phosphoglycolipid				PGL				PGL		PGL
Polar lipid	L1–3, 5–7	L1–4	L1, 3–5, 7	L1–3	L5, 6	L1–6		L1–3, 5, 6	L1, 2, 6–8	L1–3, 5
Aminophosphoglycolipid							APGL1, 2			APGL1, 2

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10
Quinone system (%):										
MK-6	3							1		
MK-7	88	63	99	100	64	67	Predominant	93	98	98
MK-8	9	36	1		36	27		6	2	2
MK-9		1								
MK-7(H ₂)						6				

and the other *Brachybacterium* strains were in excellent agreement with data from the literature (Collins *et al.*, 1988; Gvozdyak *et al.*, 1992; Takeuchi *et al.*, 1995), but the quinone system of strain C3H-21^T distinguishes it from *B. alimentarium* and *B. sacelli*, which were reported to have a quinone system with menaquinones MK-7 and MK-8 in a ratio of 2:1 (Schubert *et al.*, 1996; Heyrman *et al.*, 2002), and from *B. fresconis*, which is the *Brachybacterium* species that also, uniquely, contains minor amounts of menaquinone MK-7(H₂) in its quinone system (Heyrman *et al.*, 2002). Polar lipids were extracted and analysed by two-dimensional TLC according to Ventosa *et al.* (1993). Strain C3H-21^T shared with other members of the genus *Brachybacterium*, which were also analysed, the genus-specific lipids diphosphatidylglycerol, phosphatidylglycerol and some unknown phospho- and glycolipids (Collins *et al.*, 1988). The detection of additional lipids (Table 1) resulted in a unique polar lipid profile which appears to be useful for differentiating strain C3H-21^T from other species of the genus *Brachybacterium* (Table 1). Cellular fatty acid methyl esters obtained from cells grown in Bacto tryptic soy broth at 28 °C by using the method of Stead *et al.* (1992) were analysed by GC (Groth *et al.*, 1996). The predominant fatty acid in strain C3H-21^T was anteiso-C_{15:0} (60.3%). Additionally, considerable amounts of iso-C_{16:0} (7.2%), iso-C_{14:0} (5.7%), anteiso-C_{17:0} (5.3%), C_{19:0} (5.3%), iso-C_{15:0} (4.5%), C_{18:1ω7c} (4.0%) and an unknown acid (4.8%) and minor amounts of C_{16:0} (1.0%) and another unknown acid (1.9%) were detected in strain C3H-21^T. This fatty acid profile is in good agreement with the major characteristics of members of the genus *Brachybacterium* (Collins *et al.*, 1988; Gvozdyak *et al.*, 1992; Takeuchi *et al.*, 1995; Schubert *et al.*, 1996; Heyrman *et al.*, 2002), although quantitative differences in the relative amounts of certain fatty acids may be useful for differentiation purposes. Mycolic acids were not detected, which were analysed according to Minnikin *et al.* (1980).

Extraction and detection of the polyamine patterns were performed as described previously (Busse & Auling, 1988; Altenburger *et al.*, 1997). The polyamine pattern of strain C3H-21^T consisted of major compounds spermine [7.0 µmol (g dry weight)⁻¹] and spermidine [5.8 µmol (g dry weight)⁻¹], and traces of putrescine and cadaverine [<0.03 µmol (g dry weight)⁻¹]. To evaluate polyamine patterns for suitability in classification of *Brachybacterium*, the type species of the genus, *B. faecium* CCM 4372^T,

was analysed for its polyamine pattern as well. This strain also contained a polyamine pattern which was characterized by the major compounds spermidine [7.5 µmol (g dry weight)⁻¹] and spermine [6.9 µmol (g dry weight)⁻¹]. Additionally, minor amounts of putrescine [0.1 µmol (g dry weight)⁻¹] and traces of cadaverine and diamino-propane were detected [<0.02 µmol (g dry weight)⁻¹]. Among the class *Actinobacteria*, similar polyamine patterns have only been detected in species of certain genera of the family *Microbacteriaceae*, namely, *Clavibacter*, *Rathayibacter* and *Curtobacterium* (Altenburger *et al.*, 1997), and genera of the family *Propionibacteriaceae*, namely, *Propioniferax*, *Friedmanniella*, *Microlunatus*, *Luteococcus* (Busse & Schumann, 1999) and *Propionibacterium* (Hamana, 1995), but these taxa can be easily distinguished from *Brachybacterium* based on their quinone systems and peptidoglycan composition. However, the overall polyamine contents in representatives of these taxa were reported to be significantly lower than those observed in strain C3H-21^T and *B. faecium* CCM 4372^T. These findings suggest that, in addition to the polyamine composition, the overall polyamine contents can be useful for discrimination of actinobacterial taxa and that species of the genus *Brachybacterium* contain a polyamine pattern which may be useful for their differentiation from other members of the class *Actinobacteria*, especially when analysed in combination with the quinone system and peptidoglycan composition.

Although strain C3H-21^T had many of the characteristics of the genus *Brachybacterium* (Collins *et al.*, 1988; Gvozdyak *et al.*, 1992; Takeuchi *et al.*, 1995; Schubert *et al.*, 1996; Heyrman *et al.*, 2002), its unique combination of chemotaxonomic characteristics indicated its separate position within the genus.

To confirm the distinct position of strain C3H-21^T within the genus *Brachybacterium*, its protein pattern, which was obtained after SDS-PAGE (Stan-Lotter *et al.*, 2002), was compared to reference strains, namely, *B. sacelli* DSM 14566^T, *B. nesterenkovii* CCM 2432, *B. rhamnosum* DSM 10240^T, *B. alimentarium* CCM 4520^T and *B. fresconis* DSM 14564^T. Cells were grown in PYES medium (g l⁻¹: 0.3% yeast extract, 0.3% peptone from casein, 0.23% Na₂-succinat; pH 7.2), harvested by centrifugation (7000 g, 5 min), washed with 0.9% (w/v) NaCl and suspended in fourfold-concentrated sample buffer (Laemmli, 1970) to a

Fig. 2. Phenogram of electrophoretic patterns of strains representing recognized *Brachybacterium* species and strain C3H-21^T, based on UPGMA analysis of the Pearson correlation coefficients (r) of the protein patterns.

ratio of 200 mg wet weight (ml buffer)⁻¹. Following four cycles of freezing–thawing, 3 volumes of water were added, and the samples were boiled for 5 min then centrifuged; supernatants were analysed by SDS-PAGE on 11 % polyacrylamide gels and Coomassie blue staining (Laemmli, 1970). Reproducibility of the protein extraction and electrophoretic procedures was confirmed by comparing duplicate extracts and by performing duplicate runs of a single extract on the same gel as well as on separate gels.

Electrophoretic protein patterns were recorded on an Umax Astra 1220 S scanner (Umax Systems) and analysed with the GELCOMP software package, Version 3.0 (Applied Maths). The software calculated the Pearson product moment correlation coefficient (r) between the samples and clustered the samples using the UPGMA. These examinations supported the separate position of strain C3H-21^T within the genus *Brachybacterium* (Fig. 2).

REP-PCR has been applied recently to assess the genomic diversity of *Brachybacterium* isolates, and completely different band patterns were reported for representatives of *B. fresconis* and *B. sacelli* (Heyrman *et al.*, 2002). Comparison of REP-PCR-generated fingerprints of strain C3H-21^T, *B. sacelli* DSM 14566^T, *B. nesterenkovi* CCM 2432, *B. rhamnosum* DSM 10240^T, *B. alimentarium* CCM 4520^T and *B. fresconis* DSM 14564^T, which was done as described by Wieser & Busse (2000), revealed unique band patterns for each strain (Fig. 1, available in IJSEM Online). Visual evaluation of the band patterns indicated that strain C3H-21^T only shared with *B. sacelli* DSM 14566^T a single band at approximately 400 bp.

Based on 16S rDNA sequence comparisons, strain C3H-21^T occupies a separate position within the genus *Brachybacterium*. This is confirmed by its unique combination of chemotaxonomic characteristics (Table 1), protein pattern (Fig. 2), REP-PCR-generated genomic fingerprint (Fig. 1, IJSEM Online) and physiological/biochemical traits (Table 1). In conclusion, we here describe a novel species of the genus *Brachybacterium*, for which we propose the name *Brachybacterium muris*.

Description of *Brachybacterium muris* sp. nov.

Brachybacterium muris (mu'ris. L. gen. n. *muris* of the mouse; the type strain was isolated from a mouse).

Cells are small coccoid to ovoid, 1.2–1.5 µm in diameter. Cells occur singly, in pairs or in agglomerates. Gram-positive and non-spore-forming. Motility is not observed. Cells grow best on sheep blood agar plates and *Brucella* broth supplemented with 8 % fetal bovine serum. Colonies on PYES agar are translucent, have light-yellow pigmentation and are circular, convex and smooth. Best growth is observed under microaerobic conditions, moderate growth is observed under aerobic conditions, but no growth occurs anaerobically. Colony diameters on sheep blood agar are 3–5 mm after 4 days incubation at 37 °C under microaerobic conditions. The temperature range for growth is 15–42 °C, with optimal growth between 25 and 37 °C; no growth at 4 °C. No growth is observed at pH 5.0. Can grow in the presence of up to 10 % NaCl. Catalase-positive. Oxidase-negative. Nitrate is reduced to nitrite. Physiological and biochemical traits are shown in Table 1. The diagnostic cell-wall diamino acid is *meso*-diaminopimelic acid and the peptidoglycan type is A31.3 (variation A4γ) containing the amino acids *meso*-diaminopimelic acid, alanine, glycine, aspartic acid and glutamic acid. Muramic acid residues are *N*-acetylated and not *N*-glycolated. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, three unknown glycolipids, two unknown phospholipids and six unidentified polar lipids. Mycolic acids are absent. The fatty acid profile consists of the predominant compound anteiso-C_{15:0} with significant amounts of iso-C_{14:0}, iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and C_{19:0}. The principal menaquinone is MK-7; MK-8 and MK-6 are present in minor amounts. Spermine and spermidine are the predominant components of the polyamine pattern.

The type strain C3H-21^T (=DSM 14566^T=CCM 7047^T) was isolated from the liver of a laboratory mouse strain.

References

- Altenburger, P., Kämpfer, P., Akimov, V. N., Lubitz, W. & Busse, H.-J. (1997). Polyamine distribution in actinomycetes with Group B peptidoglycan and species of the genera *Brevibacterium*, *Corynebacterium*, and *Tsukamurella*. *Int J Syst Bacteriol* **47**, 270–277.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Buczolits, S., Rosengarten, R., Hirt, R. & Busse, H.-J. (2001). Classification of a *Brevundimonas* strain detectable after PCR with a *Helicobacter*-specific primer pair. *Syst Appl Microbiol* **24**, 368–376.
- Buczolits, S., Denner, E. B. M., Vybiral, D., Wieser, M., Kämpfer, P. & Busse, H.-J. (2002). Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. *Int J Syst Evol Microbiol* **52**, 445–456.

- Busse, H.-J. & Auling, G. (1988). Polyamine patterns as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Busse, H.-J. & Schumann, P. (1999). Polyamine profiles within genera of the class *Actinobacteria* with LL-diaminopimelic acid in the peptidoglycan. *Int J Syst Bacteriol* **49**, 179–184.
- Collins, M. D., Brown, J. & Jones, D. (1988). *Brachybacterium faecium* gen. nov., sp. nov., a coryneform bacterium from poultry litter. *Int J Syst Bacteriol* **38**, 45–48.
- de Waard, R., Claassen, E., Bokken, G. C. A. M., Buiting, B., Garssen, J. & Vos, J. G. (2003). Enhanced immunological memory responses to *Listeria monocytogenes* in rodents, as measured by delayed-type hypersensitivity (DTH), adoptive transfer of DTH, and protective immunity, following *Lactobacillus casei* Shirota ingestion. *Clin Diagn Lab Immunol* **10**, 59–65.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Department of Genetics, University of Washington, Seattle, USA.
- Fox, J. G., Dewhirst, F. E., Tully, J. G., Paster, B. J., Yan, L., Taylor, N. S., Collins, M. J., Jr, Gorelick, P. L. & Ward, J. M. (1994). *Helicobacter hepaticus* sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. *J Clin Microbiol* **32**, 1238–1245.
- 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.
- Groth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B. & Augsten, K. (1997). *Bogoriella caseilytica* gen. nov., sp. nov., a new alkaliphilic actinomycete from a soda lake in Africa. *Int J Syst Bacteriol* **47**, 788–794.
- Gvozdyak, O. R., Nogina, T. M. & Schumann, P. (1992). Taxonomic study of the genus *Brachybacterium*: *Brachybacterium nesterenkovi* sp. nov. *Int J Syst Bacteriol* **42**, 74–78.
- Hamana, K. (1995). Polyamine distribution patterns in coryneform bacteria and related gram-positive eubacteria. *Annu Rep Coll Med Care Technol Gunma Univ* **16**, 69–77.
- Heyrman, J., Balcaen, A., De Vos, P., Schumann, P. & Swings, J. (2002). *Brachybacterium fresconis* sp. nov. and *Brachybacterium sacelli* sp. nov., isolated from deteriorated parts of a medieval wall painting of the chapel of Castle Herberstein (Austria). *Int J Syst Evol Microbiol* **52**, 1641–1646.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr* **188**, 221–233.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sakao, Y., Takeda, K., Tsutsui, H., Kaisho, T., Nomura, F., Okamura, H., Nakanishi, K. & Akira, S. (1999). IL-18-deficient mice are resistant to endotoxin-induced liver injury but highly susceptible to endotoxin shock. *Int Immunol* **11**, 471–480.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schubert, K., Ludwig, W., Springer, N., Kroppenstedt, R. M., Accolas, J.-P. & Fiedler, F. (1996). Two coryneform bacteria isolated from the surface of French Gruyère and Beaufort cheeses are new species of the genus *Brachybacterium*: *Brachybacterium alimentarium* sp. nov. and *Brachybacterium tyrofermentans* sp. nov. *Int J Syst Bacteriol* **46**, 81–87.
- Stan-Lotter, H., Pfaffenhueimer, M., Legat, A., Busse, H.-J., Radax, C. & Gruber, C. (2002). *Halococcus dombrowskii* sp. nov., an archaeal isolate from a Permian alpine salt deposit. *Int J Syst Evol Microbiol* **52**, 1807–1814.
- Stead, D. E., Sellwood, J. E., Wilson, J. & Viney, I. (1992). Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* **72**, 315–321.
- Takeuchi, M., Fang, C.-X. & Yokota, A. (1995). Taxonomic study of the genus *Brachybacterium*: proposal of *Brachybacterium conglomeratum* sp. nov., nom. rev., *Brachybacterium paraconglomeratum* sp. nov., and *Brachybacterium rhamnosum* sp. nov. *Int J Syst Bacteriol* **45**, 160–168.
- Tindall, B. J. (1990). A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tsuji, H., Harada, A., Mukaida, N. & 7 other authors (1997). Tumor necrosis factor receptor p55 is essential for intrahepatic granuloma formation and hepatocellular apoptosis in a murine model of bacterium-induced fulminant hepatitis. *Infect Immun* **65**, 1892–1898.
- Ventosa, A., Marquez, M. C., Kocur, M. & Tindall, B. J. (1993). Comparative study of “*Micrococcus* sp.” strains CCM 168 and CCM 1405 and members of the genus *Salinicoccus*. *Int J Syst Bacteriol* **43**, 245–248.
- Wieser, M. & Busse, H.-J. (2000). Rapid identification of *Staphylococcus epidermidis*. *Int J Syst Evol Microbiol* **50**, 1087–1093.
- 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.