Identification of a bacterial strain isolated from the liver of a laboratory mouse as *Microbacterium paraoxydans* and emended description of the species *Microbacterium paraoxydans* Laffineur et al 2003

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Abstract A rod-shaped, Gram-positive bacterial strain, designated C57-33, was isolated from the liver of the laboratory mouse strain C57Bl/6J and characterised by a polyphasic approach. 16S rRNA gene sequence similarity placed strain C57-33 in the genus *Microbacterium* with *Microbacterium paraoxydans* CF36^T as the next relative (99.9 % sequence similarity). Major fatty acids ai-C_{15:0}^a, i-C_{16:0} and ai-C_{17:0} and peptidoglycan type B2β with ornithine as the diagnostic cell-wall diamino acid and glycolyl residues were in agreement with the description of *Microbacterium paraoxydans*. The quinone system of C57-33 (major menaquinones MK-12 and MK-11) and polar lipid profile (major polar lipids diphosphatidyl glycerol, phosphatidyl glycerol and two unknown glycolipids) were in accordance with those of *Microbacterium paraoxydans* strains CF36^T, CF7 and CF40 which were analysed in this study as well. The results of biochemical/physiological characterisation,

DNA-DNA hybridization, MALDI-TOF mass spectrometry of cell extracts and comparison of protein patterns after SDS-PAGE demonstrated that our isolate C57-33 (= DSM 15461) is a strain of the species *Microbacterium paraoxydans*. Based on new characteristics such as quinone system, polar lipid profile and physiological traits analysed for strain C57-33, the type strain of *Microbacterium paraoxydans* and some additional strains an emended description of the species *Microbacterium paraoxydans* is provided.

Keywords *Microbacterium paraoxydans* · 16S rDNA sequence · chemotaxonomy · biochemical/physiological traits · emended description

Introduction

The genus *Microbacterium*, as it was redefined by Takeuchi and Hatano [1], encompasses a set of species, which share high 16S rRNA sequence similarities (>95 %) but exhibit several variations in chemotaxonomic traits such as peptidoglycan type and quinone system. For instance species of the genus contain a peptidoglycan based on lysine (B1α or B1β) or ornithine (B2α or B2β) and quinone systems with the major compounds MK-10 and MK-11, MK-11 and MK-12, MK-12, MK-12 and MK-13, MK-13 or MK-13 and MK-14². This variability is remarkable because the two markers are considered to be quite conserved and similar differences among species of a single genus have been rarely reported. Recently, a study on house-keeping genes has identified several clusters [3] among species of

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the genus which at least in some cases are confirmed by peptidoglycan types and quinone systems. Hence, analyses of peptidoglycan type and quinone is highly desirable in phenotyping of novel *Microbacterium* species.

In a study concerning the bacteriological biodiversity in the liver of different laboratory mice strains (C3H/He and C57Bl/6J), among others, a yellow-pigmented strain was isolated on sheep blood agar at 37°C under microaerobic conditions which was designated C57-33. In order to find a taxonomic home for this strain it was analysed by a complex set of methods and compared to reference strains.

Materials and methods

Morphological and physiological characterization

Strain C57-33 was isolated from the liver of a laboratory mouse strain on sheep blood agar at 37 °C under microaerobic conditions as described recently [4,5]. Colonies were subcultivated on PYES-agar (1^l: 3 g yeast extract, 3 g peptone from casein, 2.3 g Na₂-succinate, 15 g agar, pH 7.2) at 28 °C for 48 h. Cell morphology and motility were examined by phase-contrast microscopy (Leitz Diaplan) from cultures of different growth stages. Gram behaviour was tested by the KOH-lysis test [6], the L-alanine-aminopeptidase test (Merck) and by staining as described by Smibert and Krieg [7]. Methods for physiological and biochemical characterization have been described recently [8, 9].

DNA extraction, 16S rDNA sequence analysis and rep-PCR

Approximately two loops of biomass were scrapped off an agar plate, suspended in 100 µl sterile water and lysed by repeated cycles of freezing in liquid nitrogen and thawing at 50 °C. After centrifugation at 13000 rpm, 5 min between 1 and 3 µl of the supernatant were used as template DNA for PCR amplification.

16S rDNA was amplified by the polymerase chain reaction (PCR) using the universal primers 27f and 1492r [10]. The PCR reaction mix contained 2 µl dNTP mix (containing 10 mM of each dNTP), 10 µl 10x polymerase buffer (Finnzymes), 2 µl of each primer (300 ng/µl) and 10 µl DNA template and 73.5 µl sterile water. 0.5 µl (2 U/µl) Dynazyme polymerase (Finnzymes) was added after an initial denaturation step. Amplification was carried out in a Primus 25 thermocycler (MWG-Biotech). The amplification program consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 2 min, annealing at 40 °C for 1.5 min and elongation at 72 °C

for 5 min. Amplification products were mixed with equal volumes of PEG solution (3 M NaCl solution containing 24 % polyethylene glycol 6000), precipitated at 37°C for 10 minutes and recovered by centrifugation at 16500 rpm. for 1 h at 4°C [11]. Purified PCR products were directly sequenced at the Service Department at the Vienna Biocenter (MIG-BASE) on a LI-COR 4000 L (LI-COR Inc., NE., USA), as outlined by Middendorf et al. [12] using the primers 27f and 1492r. The derived sequence was compared to corresponding sequences using FASTA [13].

For ERIC-, BOX- and REP-PCR, which were carried out as described previously [14], the following primers (100 pmol each) were employed: ERIC-PCR (ERIC1R: 5'-ATGTAAGCTCCTGGGGATTAC-3', ERIC2: 5'-AAGTAAGTGACTGGGGTGAGCG-3'), REP-PCR (REP1R-I: 5'-IIICGICGICATCIGGC-3', REP2-I: 5'-ICGICTTATCIGGCCTAC-3') and BOX-PCR (BOXA1R: 5'-CTACGGCAAGGCGACGCTGACG-3'). An initial denaturation step at 95 °C for 7 min was common to all PCRs, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 44 °C, 52 °C or 53 °C for 1 min for REP-, ERIC- or BOX-PCR, extension at 65 °C for 8 min and a final extension step (65 °C for 16 min). Between 4 to 8µl of PCR product were separated by agarose gel electrophoresis at room temperature on 1.5 % agarose gels, stained with ethidium bromide and photographed on a UV transilluminator. Fingerprints were compared visually.

RiboPrinting

RiboPrinting was performed with the automated RiboPrinter® Microbial Characterization System (Qualicon-DuPont) by using the restriction enzyme *PvuII* [15]. The band patterns were compared by using the BioNumerics software (version 3.0, Applied Maths). Clustering was carried out by the unweighted pair group method with arithmetic averages (UPGMA) based on the Pearson's correlation coefficient (optimization coefficient 1.2 %).

MALDI-TOF mass spectrometry

Sample preparation for matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was carried out according to the ethanol/formic acid extraction protocol recommended by Bruker Daltonics (<http://clinprot.bdal.de>): approximately 10 mg biomass from agar cultures was suspended in 300 µl water by careful mixing. Then the suspension was mixed with 900 µl ethanol. The biomass was collected by centrifugation and the pellet was re-suspended in 50 µl 70 % formic acid. The suspension was mixed with 50 µl acetonitrile. Immediately after

centrifugation, the supernatant was removed and aliquots of 1.5 µl were placed on the marked spots of a stainless-steel target plate. After air-drying 1.5 µl matrix solutions (saturated solution of α -cyano-hydroxy-cinnamic acid in 50 % aqueous acetonitrile containing 2.5 % trifluoroacetic acid) per spot were applied. MALDI-TOF MS was conducted using a Microflex L20 mass spectrometer (Bruker Daltonics) equipped with a N₂ laser. All spectra were recorded in linear, positive ion mode. The acceleration voltage was 20 kV. Spectra were collected as a sum of 500 shots across a spot. A mass range of 2000–20,000 *m/z* was used for analysis. Main spectra were calculated from 16 spectra per strain and used for construction of a score oriented dendrogram by using the BioTyper software (version 1.1, Bruker Daltonics).

Analysis of polar lipids, quinones, protein patterns, fatty acids and peptidoglycan

Polar lipids and quinones were analysed as described by Ventosa et al. [16] and Tindall [17]. 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. [18] were analysed by gas chromatography [19]. Analyses of the protein patterns were done as described previously [20]. Purified cell wall preparations were obtained by the method of Schleifer and Kandler [21] and the amino acids and peptides in peptidoglycan hydrolysates were analysed as described previously [22].

DNA-DNA hybridization

DNA–DNA hybridizations were carried out following the method described by Ziemke et al. [23].

Results and Discussion

Genomic analyses

PCR-amplification of the 16S rDNA of strain C57-33 resulted in a fragment of 1435 bases (positions 8 - 1461, *E. coli* numbering [24]). Sequence comparisons showed that strain C57-33 shared the highest similarities with *Microbacterium paraoxydans* CF36^T (99.9 %), *Microbacterium oxydans* DSM 20578^T (99.5 %), *Microbacterium luteolum* IFO 15074^T (99.4 %), *Microbacterium liquefaciens* DSM 20638^T (99.2 %) and *Microbacterium saperdae* IFO 15038^T (99.2 %). Sequence similarities to other species of the genus *Microbacterium* were in the range of 95.0–98.9 %. Majority of signature nucleotides used for the description of the genus [1] were also detected in the 16S rDNA sequence of

isolate C57-33. Some differences, however, were found: positions 838 (guanosine instead of uridine), 856 (uridine instead of adenosine) and 1386 (adenosine instead of uridine). Identical deviations were also detected in the 16S rDNA sequence of *M. paraoxydans* CF36^T. Both, sequence similarities and presence of unusual signatures indicated affiliation of strain C57-33 with *M. paraoxydans*. To confirm this indicated relatedness DNA:DNA hybridization was done and 100 % DNA-relatedness between C57-33 and *M. paraoxydans* CF36^T demonstrated unambiguously that they belong to the same species.

In order to find alternatives to identify strains of this clinical relevant species *M. paraoxydans* we employed several rapid methods which sometimes are useful to replace DNA-DNA hybridizations. The strains CF36^T, CF7, CF34, CF40, CF128 and CF130, which were considered in the original description of *M. paraoxydans* [25], C57-33, *M. oxydans* DSM 20578^T, *M. liquefaciens* DSM 20638^T and *M. saperdae* IFO 15038^T were subjected to REP-, ERIC- and BOX-PCR but the resulting genomic fingerprints did not exhibit any band specific for all strains of *M. paraoxydans* including strain C57-33 (results not shown). However, this result is not surprising because these genomic fingerprinting techniques are often more suitable for detection of clonal relationships and our results indicate that these approaches in fact are more useful in studying the epidemiology of a particular strain. Also Riboprint analyses using *Pvu*II revealed that the *M. paraoxydans* strains displayed strain-specific patterns though containing a common band at ca. 9 kb (Fig. 1). However, a band of similar size was also detected in *M. saperdae* DSM 20169^T. Some similarities were observed in the patterns of strains CF128 and DSM 15021 (CF40) and in the patterns of strains CF34 and DSM 15020 (CF7), respectively. The strains DSM 15019^T (CF36^T), CF130 and C57-33 were characterized by more individual band patterns.

Phenotyping

In order to examine phenotypic congruence of our isolate C57-33 with the other six strains *M. paraoxydans* [25] and to identify differentiating traits these strains and type strains of related species were subjected to chemotaxonomic and physiological characterisations.

Recent studies proved the usefulness of MALDI-TOF mass spectra for classification and identification of bacteria [26,27] and demonstrated that MALDI-TOF MS is able to reflect the phylogenetic relationship of microorganisms in agreement with results of sequence analyses of conservative genes [28]. The dendrogram constructed from main spectra of *M. paraoxydans* strains, of *Microbacterium* type strains clustering together with *M. paraoxydans* in a

phylogenetic tree according to Schippers et al. [29] and of the only moderately related *Microbacterium resistens* DSM 11986^T demonstrated that the strains C57-33, CF128, CF34, CF130, DSM 15020 (= CF7) and DSM 15021 (= CF40) share a separate cluster with *M. paraoxydans* DSM 15019^T (= CF36^T) (Fig. 2). This result confirms that the six strains belong to the species *M. paraoxydans* and underpins the high potential of MALDI-TOF MS for classification and identification of microbacteria.

Microbacteria are well-known for their significant variability among species [1, 2, 30, 31] whereas information on polar lipid profiles within the genus is limited. Since the description of *M. paraoxydans* is lacking information concerning quinone system and polar lipids four strains of *M. paraoxydans* (C57-33, CF36^T, CF7 and CF40) as well as type strains of related species were subjected to analysis for these chemotaxonomic traits. For complementation of the chemotaxonomic characterization strain C57-33 was

Fig. 1. *Pvu*II RiboPrint patterns of strain C57-33, *M. paraoxydans* strains and type strains of highly related *Microbacterium* species as determined by 16S rRNA gene sequence comparisons. The analysis was performed with the RiboPrinter microbial characterization system (Qualicon Du Pont) and the RiboPrint pattern was analysed with the BioNumerics software (Version 3.0, Applied Maths).

Fig. 2. Score oriented dendrogram generated by the BioTyper software (version 1.1, Bruker Daltonics) showing the similarity of MALDI-TOF mass spectra of cell extracts of strain C57-33, *M. paraoxydans* strains, phylogenetically related *Microbacterium* type strains and *M. resistens* DSM 11986^T.

also subjected to analyses of peptidoglycan type and fatty acid profile.

C57-33 contained the major menaquinones MK-12 and MK-11 and minor amounts of menaquinones MK-13 and MK-10. Strains CF36^T, CF7 and CF40 showed unexpected highly similar quinone systems only slightly varying from the relative amounts detected in strain C57-33 (Table 1). Re-investigation of the quinone systems of the type strains of *M. oxydans*, *M. liquefaciens*, *M. saperdae* and *M. luteolum* revealed some significant differences in the relative amount of certain quinones which appeared to be useful for differentiation among each other and from *M. paraoxydans* as well (Table 1). This assumption needs to be confirmed by analyses of additional strains of the reference species. However, these data indicate that the quinone system can be useful for differentiation between closely related *Microbacterium* species and the variation between different strains, as demonstrated for *M. paraoxydans*, appears to be rather limited when grown under identical conditions (medium composition, temperature). Thus, quinone analyses should be routinely analysed in detail (% of the total), when novel species of the genus are described and not only as ‘major’ and ‘minor’ compounds.

Strains C57-33, CF36^T (Fig. 3A), CF7 and CF40 shared with *M. oxydans*_NCIB 9944^T, *M. liquefaciens*_ATCC 43647^T, *M. saperdae*_DSM 20169^T and *M. luteolum*_DSM 20143^T (Fig. 3B) the genus-specific lipids diphosphatidyl

glycerol, phosphatidyl glycerol, one unknown glycolipid (GL1), an unknown phospholipid and three unidentified polar lipids (Table 1) [31, 32]. The detection of one additional unknown glycolipid (GL2), and two unidentified polar lipids resulted in a unique polar lipid profile which distinguished strains C57-33, CF36^T, CF7 and CF40 from next related species. Another unique profile was detected in *M. luteolum*_DSM 20143^T characterized by the presence of another unknown glycolipid (GL3). In contrast, no unique characteristics were detected in the profiles of *M. oxydans*_DSM 20578^T, *M. liquefaciens*_DSM 20638^T and *M. saperdae*_DSM 20169^T. These results demonstrate that a detailed analysis of polar lipids can be useful for discrimination between closely related *Microbacterium* species.

The fatty acid profile of C57-33 was predominated by the presence of ai-C_{15:0} (50.8 %). Additionally, high amounts of i-C_{16:0} (19.8 %) and ai-C_{17:0} (19.1 %), moderate amounts of i-C_{15:0} (6.0 %) and minor amounts of C_{16:0} (2.0 %), i-C_{17:0} (1.2 %) and i-C_{14:0} (1.1 %) were detected in C57-33. This fatty acid profile is in good accordance with cellular fatty acids of *M. paraoxydans* strains [25].

The peptidoglycan of strain C57-33 contained glycolyl residues, ornithine (Orn), glycine (Gly), alanine (Ala), glutamic acid (Glu), hydroxyglutamic acid (Hyg) and homoserine (Hsr). From these data we concluded that the peptidoglycan type is B2β [21], D-Glu(Hyg) → Gly → D-Orn with Gly at position 1 and L-Hsr at position 3 of

Fig. 3. Two-dimensional thin-layer chromatogram of polar lipids of (A) *Microbacterium paraoxydans* CF36^T and (B) *Microbacterium luteolum* DSM 20143^T. DPG: diphosphatidylglycerol; PG: phosphatidylglycerol; PL1: unknown phospholipid; GL1-3: unknown glycolipids; L1-5: unknown polar lipids.

Table 1. Characteristics of strain C57-33 and *M. paraoxydans* strains (CF36^T, CF7, CF34, CF40, CF128, CF130) differentiating them from their phylogenetic neighbours with which they share the quinone system with the predominant compounds MK-11 and MK-12[2].

Organism Characteristics	C57-33	<i>M. paraoxydans</i> (n = 6) [§]	<i>M. oxydans</i> NCIB 9944 ^T	<i>M. liquefaciens</i> ATCC 43647 ^T	<i>M. saperdae</i> DSM 20169 ^T	<i>M. luteolum</i> DSM 20143 ^T
Urease production	-	-	-	+	+	+
H ₂ S production	-	-	+	+	+	+
Nitrate reduction	-	-	-	+	-	+
Growth at:						
4 °C	-	-	+	+	+	(+)
37 °C	+	+	+	+ [§]	-	-
42 °C	+	+	-	-	-	-
Growth at:						
pH 10.0	+	+	+	+	-	-
Growth in the presence of:						
7 % NaCl	+	+	+	(+)	-	-
Hydrolysis of:						
Casein	+	+	+	-	-	-
Starch	+	+	-	-	+	-
Tween 20	+	+	+	-	+	-
Acid production from:						
D-Arabinose	-	-	-	-	(+)	-
L-Arabinose	-	-	+	+	+	-
DL-Lactose	-	-	-	-	+	-
Melibiose	(+)	v ^{&}	+	+	(+)	-
Raffinose	-	-	(+)	(+)	+	-
L-Rhamnose	(+)	v ^{&}	-	+	-	(+)
D-Ribose	(+)	(+)	+	-	-	-
D-Xylose	-	v ^{&}	+	+	+	+
quinone system						
MK-10	7 %	3-7 %	14 %	10 %	-	1 %
MK-11	31 %	33-36 %	19 %	46 %	34 %	19 %
MK-12	52 %	52-57 %	67 %	44 %	66 %	63 %
MK-13	10 %	4-6 %	-	-	-	17 %
distinguishing polar lipid(s)	GL2, PL1, L2, L5	GL2, PL1, L2, L5	-	-	-	GL3

+, positive; (+), weakly positive; -, negative; v, variable.

Abbreviation: MK, menaquinone; DPG, diphosphatidyl glycerol; PG, phosphatidyl glycerol; GL1, GL2, GL3, unknown glycolipids; PL1, unknown phospholipid; L1, L2, L3, L4, unknown polar lipids.

[§]Result not in line with those of Yokota et al.[32]

[§] Only strains CF36^T, CF7 and CF40 were subjected to analyses of quinone systems and polar lipid profiles.

[&]The type strain reacts negatively.

All of the *Microbacterium* strains were positive in tests for catalase, decomposition of aesculin and acid production from D-fructose, D-galactose, D-glucose, maltose, D-mannose and sucrose and grew well from pH 5.0 to pH 9.0, in the temperature range from 15°C to 28°C and in the presence of 0 to 5 % NaCl (w/v). All strains were negative in tests for oxidase, indole formation and Voges-Proskauer, decomposition of tween 80 and acid production from D-sorbit.

Fig. 4. Two-dimensional thin-layer chromatogram of polar lipids of (A) *Microbacterium paraoxydans* CF36^T and (B) *Microbacterium luteolum* DSM 20143^T. DPG: diphosphatidylglycerol; PG: phosphatidylglycerol; PL1: unknown phospholipid; GL1-3: unknown glycolipids; L1-5: unknown polar lipids.

the peptide subunit (type B6; <http://www.dsmz.de/species/murein.htm>). This feature is in agreement with the peptidoglycan type of representatives of *M. paraoxydans* [25].

Analyses of the protein patterns obtained after SDS polyacrylamide gel electrophoresis substantiated affiliation of the seven *M. paraoxydans* strains C57-33, CF36^T, CF7, CF34, CF40, CF128 and CF130 to a single species (Fig. 4).

Furthermore, the complete set of *M. paraoxydans* strains (C57-33, CF36^T, CF7, CF34, CF40, CF128 and CF130) were examined for physiological characteristics. Gram-behaviour in Gram-staining, KOH-test and aminopeptidase-test was positive. Other characteristic features of strain C57-33 and the *M. paraoxydans* strains CF36^T, CF7, CF34, CF40, CF128 and CF130 are summarised in Table 1 and in the emended species description below. Lack of any deviation from the physiological/biochemical profile recorded for *M. paraoxydans* based on the examination of six strains confirmed affiliation of strain C57-33 with this species.

From congruence in the quinone system, polar lipid and fatty acid profiles, protein patterns, physiological characteristics, clustering by MALDI-TOF mass spectra of cell extracts and DNA-DNA-relatedness it becomes evident that the isolate C57-33 is a strain of *M. paraoxydans*. Furthermore, our data demonstrate that in addition to the results

provided by Laffineur et al. [25] *M. paraoxydans* can be distinguished from its phylogenetic relatives *M. oxydans* DSM 20578^T, *M. liquefaciens* DSM 20638^T, *M. saperdae* IFO 15038^T and *M. luteolum* IFO 15074^T based on other chemotaxonomic and physiological traits (Table 1) enhancing a reliable identification. Our examination of several strains of *M. paraoxydans* including CF36^T has provided new knowledge on characteristics of this species and thus an emended description of *M. paraoxydans* is given below.

Emended description of *Microbacterium paraoxydans* Laffineur et al. 2003

In addition to the properties given in the original species description, this species has the following characteristics. Growth is observed under aerobic and microaerobic conditions but no growth occurs anaerobically. Colony diameters on PYES-agar are 4–6 mm after 48 hours of incubation at 28 °C under aerobic conditions. The temperature range for growth on PYES-agar is 15 °C to 42 °C, with optimal growth between 25 °C and 37 °C; no growth at 4 °C. Additional new physiological and biochemical traits are listed in Table 1. The polar lipid profile is composed of diphosphatidyl glycerol, phosphatidyl glycerol, two unknown glycolipids, one unknown phospholipid and four unidentified polar lipids. The principle menaquinones are MK-12 (52–57%) and MK-11 (31–36%); MK-13 (4–10%) and MK-10 (3–7%) are present in minor amounts. The peptidoglycan type is B2β containing the amino acids D-ornithine, glycine, alanine, glutamic acid, hydroxyglutamic acid and homoserine. Muramic acid residues are glycolylated.

Isolated from various human body sites and from the liver of a laboratory mouse. The type strain is CF36^T (DSM 15019^T, CCUG 46601^T). Other strains accessible from culture collections are CF7 (DSM 15020, CCUG 46602), CF40 (DSM 15021, CCUG 46603) and C57-33 (DSM 15461). The 16S rDNA sequence of strain C57-33 (= DSM 15461) has been deposited at the EMBL database under accession number aj581908.

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References

1. Takeuchi M and Hatano K (1998) Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins et al. in a redefined genus *Microbacterium*. Int J Syst Bacteriol 48:973–982

2. Behrendt U, Ulrich A and Schumann P (2001) Description of *Microbacterium foliorum* sp. nov. and *Microbacterium phyllosphaerae* sp. nov., isolated from the phyllosphere of grasses and the surface litter after mulching the sward, and reclassification of *Aureobacterium resistens* (Funke *et al.* 1998) as *Microbacterium resistens* comb. nov. Int J Syst Evol Microbiol 51:1267–1276
3. Richert K, Brambilla E and Stackebrandt E (2007) The phylogenetic significance of peptidoglycan types: Molecular analysis of the genera *Microbacterium* and *Aureobacterium* based upon sequence comparisons of *gyrB*, *rpoB*, *recA*, and 16S rRNA genes. System Appl Microbiol 30:102–108
4. Fox JG, Dewhirst FE, Tully JG, Paster BJ, Yan L, Taylor NS, Collins MJ jr, Gorelick PL and Ward JM (1994) *Helicobacter hepaticus* sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. J Clin Microbiol 32:1238–1245
5. Buczolits S, Schumann P, Weidler G, Radax C and Busse H-J (2003) *Brachybacterium muris* sp. nov., isolated from the liver of a laboratory mouse strain. Int J Syst Evol Microbiol 53:1955–1960
6. Moaledji K (1986) Comparison of gram-staining and alternate methods, KOH test and aminopeptidase activity in aquatic bacteria: their application to numerical taxonomy. J Microbiol Meth 5:303–310
7. Smibert RM and Krieg NR (1994) Phenotypic characterisation, In: Gerhardt P, Murray RGE, Woods WA and Krieg NR (ed.), Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington, 607–654
8. Buczolits S, Denner EBM, Vybiral D, Wieser M, Kämpfer P and Busse H-J (2002) Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. Int J Syst Evol Microbiol 52:445–456
9. Zlamala C, Schumann P, Kämpfer P, Rossello-Mora R, Lubitz W and 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
10. Lane DJ (1991) 16S/23S rRNA sequencing. In: Nucleic Acid Techniques in Bacterial Systematics, pp. 115–175. Edited by E. Stackebrandt and M. Goodfellow. Chichester: Wiley
11. Wieser M, Schumann P, Martin K, Altenburger P, Burghardt J, Lubitz W and Busse HJ (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
12. Middendorf LR, Bruce JC, Bruce RC, Eckles RD, Grone DL, Roemer SC, Sloniker GD, Steffens DL, Sutter SL, Brumbaugh JA and Patonay G (1992) Continuous, on-line DNA sequencing using a versatile infrared laser scanner}electrophoresis apparatus. Electrophoresis 13:487–494
13. Pearson W and Lipman DJ (1988) Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85:2444–2448
14. Wieser M and Busse H-J (2000) Rapid identification of *Staphylococcus epidermidis*. Int J Syst Evol Microbiol 50: 1087–1093
15. Bruce JL (1996) Automated system rapidly identifies and characterizes microorganisms in food. Food Technol. 50:77–81
16. Tindall BJ (1990) A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. System Appl Microbiol 13:128–130
17. Ventosa A, Marquez MC, Kocur M and Tindall BJ (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
18. Stead DE, Sellwood JE, Wilson J and 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
19. Groth I, Schumann P, Weiss N, Martin K and Rainey FA (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
20. Altenburger P, Kämpfer P, Makristathis A, Lubitz W and Busse H-J (1996) Classification of bacteria isolated from a medieval wall painting. J Biotechnol 47:39–52
21. Schleifer KH and Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477
22. Groth I, Schumann P, Rainey FA, Martin K, Schuetze B and 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
23. Ziemke F, Höfle MG, Lalucat J and Rossello-Mora R (1998) Reclassification of *Shewanella putrefaciens* Owen’s genomic group II as *Shewanella baltica* sp. nov. Int J Syst Bacteriol 48:179–186
24. Brosius J, Palmer ML, Kennedy PJ and Noller HF (1978) Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc Natl Acad Sci USA 75: 4801–4805
25. Laffineur K, Avesani V, Cornu G, Charlier J, Janssens M, Wauters G and Delmée M (2003) Bacteremia due to a novel *Microbacterium* species in a patient with leukemia and description of *Microbacterium paraoxydans* sp. nov. J Clin Microbiol 41:2242–2246
26. Maier T and Kostrzewa M (2007) Fast and reliable MALDI-TOF MS-based microorganism identification. Chemistry Today 25:68–71
27. Kroppenstedt RM, Mayilraj S, Wink JM, Kallow W, Schumann P, Secondini C and Stackebrandt E (2005) Eight new species of the genus *Micromonospora*, *Micromonospora citrea* sp. nov., *Micromonospora echinaurantiaca* sp. nov., *Micromonospora echinofusca* sp. nov., *Micromonospora fulviviridis* sp. nov., *Micromonospora inyonensis* sp. nov., *Micromonospora peucetia* sp. nov., *Micromonospora sagamiensis* sp. nov., and *Micromonospora viridifaciens* sp. nov. System Appl Microbiol 28:328–339
28. Stackebrandt E, Päuker O and Erhard M (2005) Grouping Myxococci (*Coralloccoccus*) strains by matrix-assisted laser desorption ionization time-of-flight (MALDI TOF) mass spectrometry: Comparison with gene sequence phylogenies. Curr Microbiol 50:71–77
29. Schippers A, Bosecker K, Spröer C and Schumann P (2005) *Microbacterium oleivorans* sp. nov. and *Microbacterium hydrocarbonoxydans* sp. nov., novel crude-oil-degrading Gram-positive bacteria. Int J Syst Evol Microbiol 55:655–660
30. Kageyama A, Takahashi Y, Matsuo Y, Kasai H, Shizuri Y, and Omura S (2007) *Microbacterium sediminicola* sp. nov. and *Microbacterium marinilacus* sp. nov., isolated from marine environments. Int J Syst Evol Microbiol 57: 2355–2359

31. Zlamala C, Schumann P, Kämpfer P, Valens M, Rossello-Mora R, Lubitz W and Busse H-J (2002) *Microbacterium aerolatum* sp. nov., isolated from the air in the 'Virgikapelle' in Vienna. Int J Syst Evol Microbiol 52: 1229–1234
32. Yokota A, Takeuchi M, Sakane T and Weiss N (1993) Proposal of six new species in the genus *Aureobacterium* and transfer of *Flavobacterium esteraromaticum* Omelianski to the genus *Aureobacterium* as *Aureobacterium esteraromaticum* comb. nov. Int J Syst Bacteriol 43:555–564