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.

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The taxonomic position of a group of coryneform bacteria isolated from the phyllosphere of grasses and the surface litter after sward mulching was investigated. On the basis of restriction analyses of 16S rDNA, the isolates were divided into two genotypes. According to the 16S rDNA sequence analysis, representatives of both genotypes were related at a level of 99.2% similarity and clustered within the genus *Microbacterium*. Chemotaxonomic features (major menaquinones MK-12, MK-11 and MK-10; predominating iso- and anteiso-branched cellular fatty acids; G+C content 64–67 mol%; peptidoglycan-type B2 β with glycolyl residues) corresponded to this genus as well. DNA–DNA hybridization studies showed a reassessment value of less than 70% between representative strains of both subgroups, suggesting that two different species are represented. Although the extensive morphological and physiological analyses did not reveal any differentiating feature for the genotypes, differences in the presence of the cell-wall sugar mannose enabled the subgroups to be distinguished from one another. DNA–DNA hybridization with type strains of closely related *Microbacterium* spp. indicated that the isolates represent two individual species, which can also be differentiated from previously described species of *Microbacterium* on the basis of biochemical features. As a result of phenotypic and phylogenetic analyses, the species *Microbacterium foliorum* sp. nov., type strain P 333/02^T (= DSM 12966^T = LMG 19580^T), and *Microbacterium phyllosphaerae* sp. nov., type strain P 369/06^T (= DSM 13468^T = LMG 19581^T), are proposed. Furthermore, the reclassification of *Aureobacterium resistens* (Funke et al. 1998) as *Microbacterium resistens* (Funke et al. 1998) comb. nov. is proposed.

Keywords: *Microbacterium foliorum* sp. nov., *Microbacterium phyllosphaerae* sp. nov., plant-associated, phenotypic and phylogenetic analysis, *Microbacterium resistens* comb. nov.

INTRODUCTION

Strains of the genus *Microbacterium* are widespread and can be isolated from different sources (Collins &

Bradbury, 1992). The above-ground parts of plants have also been reported as a habitat of such bacteria possessing the group B type of peptidoglycan. Thompson et al. (1993) and Legard et al. (1994)

The EMBL accession numbers for the 16S rDNA sequences of *Microbacterium foliorum* P 333/02^T (= DSM 12966^T = LMG 19580^T) and *Microbacterium phyllosphaerae* P 369/06^T (= DSM 13468^T = LMG 19581^T) are AJ249780 and AJ277840, respectively.

isolated strains of the species *Microbacterium lacticum*, *Microbacterium liquefaciens* and *Microbacterium saperdae* from the phyllospheres of sugar beet (*Beta vulgaris*) and spring wheat (*Triticum aestivum*). McInroy & Kloepper (1995) detected strains of *Microbacterium* spp. as well as *Aureobacterium* spp. living endophytically in sweet corn (*Zea mays*) and cotton (*Gossypium hirsutum*). However, the species of the genus *Aureobacterium* were accommodated in the genus *Microbacterium* as a consequence of a thorough taxonomic reinvestigation of these organisms (Takeuchi & Hatano, 1998a). Coincidental with this reclassification of *Aureobacterium* species, a new species of this genus, *Aureobacterium resistens*, was described (Funke *et al.*, 1998) and therefore could not be included in the study of Takeuchi & Hatano (1998a). Although *A. resistens* displays all the taxonomic characteristics of the redefined genus *Microbacterium*, the reclassification of *A. resistens* as *Microbacterium resistens* comb. nov. is still pending.

In the course of studying the composition of phyllosphere microbial communities of grasses and the microbes in decaying surface litter after sward mulching, a group of bacterial isolates with characteristics similar to those of the genus *Microbacterium* was also found. Conventional physiological and morphological tests did not permit an affiliation at the species level. Consequently, 16S rDNA sequence analysis and DNA–DNA hybridization studies were necessary to determine the phylogenetic relationships of these bacteria. Thus, 23 strains were randomly selected from different plots and/or sampling dates for phenotypic and phylogenetic characterization to determine their taxonomic position unambiguously.

METHODS

Bacterial strains and cultivation. The bacterial strains examined in this study were isolated from grasses and surface litter, as described previously (Behrendt *et al.*, 1997). The histories and corresponding numbers of the isolates and type strains of *Microbacterium* spp. and *A. resistens* used for comparative studies are listed in Table 1. General laboratory cultivation was performed on nutrient agar II (SIFIN) or in nutrient broth II (SIFIN) at 25 °C unless otherwise stated. Stocks of all cultures were maintained at –79 °C, using the Microbank storage system (Pro-Lab Diagnostics).

Morphological, physiological and biochemical characterization. Cell morphology was determined by light microscopy after 24 and 72 h cell growth. The motility of cells was tested by using the hanging-drop method and staining of flagella, according to the method described by Rudolph & Marvidis (1990). The Gram reaction was tested by using the classical staining procedure, as described by Süßmuth *et al.* (1987). The rapid KOH string test (Ryu, 1938), growth on MacConkey agar (Merck), and the test for L-alanine aminopeptidase (Bactident test strips; Merck) were also applied. Most tests for characterizing the biochemical profiles of studied strains were performed as described previously (Behrendt *et al.*, 1999). The production of acetoin (the Voges–Proskauer reaction) was determined according to Süßmuth *et al.* (1987). The hydrolysis of Tween 60, Tween

80, and starch was assayed according to Sands (1990). Growth on TTC and CNS medium was tested according to Vidaver & Davis (1994). The capacity for anaerobic growth was tested using Anerocult A (Merck). API 20NE (bio-Mérieux) and the API 50CH gallery using the API CHE suspension medium (bio-Mérieux) were applied to determine additional physiological and biochemical characteristics.

Cluster analysis of physiological features was performed by using the unweighted pair group arithmetic average-linkage algorithm method, which was based on Pearson correlation or squared Euclidean distances (Sneath & Sokal, 1973).

Determination of chemotaxonomic characteristics. Methods for the determination of peptidoglycan structure, menaquinone patterns and DNA base composition have been described previously (Groth *et al.*, 1999). The peptidoglycan structure was elucidated by two-dimensional ascending TLC of amino acids and peptides in cell-wall hydrolysates on cellulose plates. Menaquinones were analysed by reversed-phase HPLC. G+C contents were determined by HPLC of nucleosides. The determination of the glycolate content was performed according to the colorimetric method of Uchida *et al.* (1999). Fatty acid methyl esters were analysed by GC as described by Stead *et al.* (1992). Sugars in cell-wall hydrolysates were analysed by TLC as described by Komagata & Suzuki (1987).

16S rDNA sequence determination and phylogenetic analysis. Restriction analyses of amplified 16S rDNA were performed as described previously (Behrendt *et al.*, 1999). For 16S rDNA sequence determination, total DNA of the representative strains P 333/02^T and P 369/06^T was obtained. Cells grown overnight in 2 ml media were washed with TE buffer (10 mM Tris/HCl, pH 8.0; 1 mM EDTA) and frozen in liquid nitrogen. The frozen cells were crushed using a glass pestil, and the lysate was resuspended in 200 µl TE buffer. Further purification was carried out using the QIAamp Blood Kit (Qiagen) according to the manufacturer's instructions. The 16S rDNA was amplified with *Pfu* DNA polymerase (Promega) and cloned into the vector PCR4Blunt-TOPO using the Zero Blunt TOPO cloning kit for sequencing (Invitrogen). A cycle sequencing protocol was applied for sequencing both complementary strands with a Li-Cor Sequencer (model 4200; MWG Biotech). The similarity values were based on pairwise comparisons of sequences. For phylogenetic analyses, the DNA sequences were aligned using the CLUSTAL W algorithm (program version 1.74; Thompson *et al.*, 1994) and the trees were constructed using the neighbour-joining and maximum-likelihood algorithms (PHYLIP computer program package, version 3.57; Felsenstein, 1993). The neighbour-joining algorithm (NEIGHBOR; Saitou & Nei, 1987) is based on a matrix of pairwise distances corrected for multiple base substitutions by the method of Kimura (1980) (DNADIST with a transition/transversion ratio of 2.0). The maximum-likelihood method (DNAML; Felsenstein, 1981) was applied with three jumbles of the dataset and without global rearrangement. The 16S rDNA sequence of *Clavibacter michiganensis* was used as the outgroup in both calculations. The neighbour-joining tree was generated using the original dataset as well as 100 bootstrap datasets to evaluate its topology.

DNA–DNA hybridization. DNA–DNA similarity was examined for P 333/02^T, P 369/06^T, P 439/06, P 449/03, *A. resistens* and the type strains of *Microbacterium* spp., as listed in Table 1, according to the spectrophotometric method used by Martin *et al.* (1997).

Table 1. Bacterial strains used in this study

Abbreviations: DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; ATCC, American Type Culture Collection, Manassas, VA, USA; CCM, Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic; CCEB, Institute of Entomology, CSAV Czechoslovak Academy of Sciences, Dept of Insect Pathology, Prague, Czech Republic; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; CIP, Collection de l'Institut Pasteur, Paris, France; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Hiroshima, Wako-shi, Japan; NCDO (= NCFB), National Collection of Food Bacteria, Aberdeen, UK; NCIB (= NCIMB = NCMB), National Collection of Industrial and Marine Bacteria, Aberdeen, UK.

Isolate no./species*	Collection number(s)	Source	Isolation date
P 206/02	–	Phyllospheres of grasses	20/04/93
P 286/08	–	Phyllospheres of grasses	22/06/93
P 315/01	–	Phyllospheres of grasses	15/06/93
P 333/02 ^T	DSM 12966 ^T , LMG 19580 ^T	Phyllospheres of grasses	29/06/93
P 334/05	–	Phyllospheres of grasses	29/06/93
P 369/06 ^T	DSM 13468 ^T , LMG 19581 ^T	Phyllospheres of grasses	13/07/93
P 375/05	–	Phyllospheres of grasses	13/07/93
P 403/11	–	Decaying grasses in the litter layer	20/07/93
P 416/01	–	Phyllospheres of grasses	27/07/93
P 421/05	–	Decaying grasses in the litter layer	27/07/93
P 423/09	–	Decaying grasses in the litter layer	03/08/93
P 434/29, P 437/09	–	Phyllospheres of grasses	10/08/93
P 438/12, P 439/06	–	Decaying grasses in the litter layer	10/08/93
P 444/21	–	Decaying grasses in the litter layer	17/08/93
P 447/09, P 448/05	–	Phyllospheres of grasses	24/08/93
P 449/03, P 449/26, P 449/29, P 450/03	–	Decaying grasses in the litter layer	24/08/93
P 469/32	–	Phyllospheres of grasses	05/10/93
<i>M. aurantiacum</i>	DSM 12506 ^T , ATCC 49090 ^T , IFO 15234 ^T , NCFB 2288 ^T	Sewage	
<i>M. aurum</i>	DSM 8600 ^T , ATCC 51345 ^T , IFO 15204 ^T	Corn-steep liquor	
<i>M. keratanolyticum</i>	DSM 8606 ^T , ATCC 35057 ^T , CCM 4375 ^T , CIP 103815 ^T , IFO 13309 ^T	Soil	
<i>M. kitamiense</i>	JCM 10270 ^T	Wastewater (sugar-beet factory)	
<i>M. lacticum</i>	DSM 20427 ^T , ATCC 8180 ^T , NCDO 747 ^T , NCIB 8540 ^T	Unknown	
<i>M. liquefaciens</i>	DSM 20638 ^T , ATCC 43647 ^T , NCIB 11509 ^T	Milk	
<i>M. luteolum</i>	DSM 20143 ^T , ATCC 51474 ^T , IFO 15074 ^T , NCIB 9568 ^T	Soil	
<i>M. maritimum</i>	DSM 12512 ^T , ATCC 19260 ^T , IFO 15779 ^T , NCIMB 1050 ^T	Sea water, marine mud	
<i>M. oxydans</i>	DSM 20578 ^T , CIP 6612 ^T , NCIB 9944 ^T	Air	
<i>A. resistens</i>	DSM 11986 ^T , CCUG 38312 ^T	Corneal ulcer	
<i>M. saperdae</i>	DSM 20169 ^T , ATCC 19272 ^T , CCEB 366 ^T	Elm borer	
<i>M. terregens</i>	DSM 20449 ^T , ATCC 13345 ^T , CCM 2634 ^T , NCIB 8909 ^T	Soil	
<i>M. testaceum</i>	DSM 20166 ^T , ATCC 15829 ^T , CCM 2299 ^T	Rice	

* Isolate nos relate to the collection at the Institute of Primary Production and Microbial Ecology, Centre for Agricultural Landscape and Land Use Research Müncheberg (ZALF), Paulinenaue, Germany.

RESULTS

Restriction analysis of 16S rDNA

To analyse the phylogenetic heterogeneity of the isolates (Table 1), 16S rRNA genes were amplified with primers described by Weisburg *et al.* (1991), resulting in a single band of approximately 1500 bp. Digestion of these PCR products (using the endonucleases *TaqI*, *HinfI*, *AhaI*, *MspI*, *SacFI* and *Sau3A*) led to identical restriction patterns for all isolates. However, one different band per restriction pattern of the 16S rDNA sequences resulted from the endonucleases *CfoI* and *HaeIII*. The isolates were nearly equally divided into two genotypes, as follows: genotype I: P 315/01, P 333/02^T, P 375/05, P 403/11, P 437/09, P 444/21, P 447/09, P 448/05, P 449/03, P 449/26, P 449/29, P 469/32; genotype II: P 206/02, P 286/08, P 334/05, P369/06^T, P 416/01, P 421/05, P 423/09, P 434/29, P 438/12, P 439/06, P 450/03). One strain of each genotype, P 333/02^T (I) and P 369/06^T (II), was chosen as a representative strain for certain investigations.

Morphological, physiological and biochemical characteristics

All of the isolates studied were Gram-positive, strictly aerobic, non-spore-forming, irregularly rod-shaped organisms. Some cells were arranged at angles, forming V-shapes, but primary branching was not observed. In older cultures, rods were shorter, but a marked rod-coccus growth cycle did not occur. Some cells were motile by means of a single polar or lateral flagellum.

The optimum growth temperature was 25 °C. At 37 °C, growth was strain-dependent, whereas none of the isolates was capable of growing at 42 °C. On solid media, colonies were circular, slightly convex with entire margins, shiny and moist. The pigment of colonies was translucent yellow and became lemon-yellow in older cultures.

Determination of the Gram reaction by means of classical staining procedures gave uncertain results for most of the strains, as they are decolorized easily and react like Gram-negative bacteria. However, the rapid

Table 2. Physiological tests showing differing results among the 23 isolates tested

Test	P 333/02 ^T (I) (DSM 12966 ^T)	P 369/06 ^T (II) (DSM 13468 ^T)	No. strains +/–	Strains showing the less common response†
Growth at 37 °C	w+	w+	15/8	P 444/21; P 449/29; P 286/08 ; P 334/05 ; P 434/29 ; P 438/12 ; P 439/06 ; P 450/03
Hydrolysis of:				
Casein	–	–	1/22	P 206/02
Tween 60	+	+	21/2	P 437/09; P 206/02
Tween 80	–	+	7/16	P 286/08 ; P 334/05 ; P 369/06^T ; P 434/29 ; P 438/12 ; P 439/06 ; P 450/03
Starch	w+	w+	22*/1	P 206/06
Oxidative acid production from glucose (API 20NE)	+	+	22/1	P 438/12
Assimilation of:				
Citrate	+	–	3/20	P 333/02 ^T ; P 444/21; P 447/09
Phenylacetate	–	+	3/20	P 447/09; P 369/06^T ; P 421/05
Acid production from (API 50CH):				
D-Arabinose	–	–	13/10	P 333/02 ^T ; P 375/05; P 403/11; P 437/09; P 447/09; P 449/26; P 286/08 ; P 369/06^T ; P 416/01 ; P 450/03
L-Arabinose	+	+	22/1	P 206/02
Methyl α-D-glucoside	–	–	19/4	P 333/02 ^T ; P 449/29; P 369/06^T ; P 434/29
L-Fucose	+	+	15/8	P 444/21; P 449/26; P 286/08 ; P 416/01 ; P 421/05 ; P 438/12 ; P 439/06 ; P 450/03
β-Gentiobiose	–	+	21/2	P 333/02 ^T ; P 449/26
Glycogen	–	–	5/18	P 441/21; P 449/26; P 416/01 ; P 421/05 ; P 438/12
Inulin	–	–	5/18	P 444/21; P 449/26; P 416/01 ; P 434/29 ; P 438/12
Inositol	–	–	2/21	P 423/09 ; P 434/29
Lactose	–	–	7/16	P 444/21; P 449/29; P 206/02 ; P 286/08 ; P 416/01 ; P 421/05 ; P 438/12
Methyl α-D-mannoside	+	+	11/12	P 333/02 ^T ; P 375/05; P 403/11; P 444/21; P 449/26; P 449/29; P 206/06 ; P 369/06^T ; P 416/01 ; P 421/05 ; P 438/12
Melibiose	–	+	13/10	P 315/01; P 333/02 ^T ; P 403/11; P 447/09; P 448/05; P 469/32; P 206/02 ; P 286/08 ; P 434/29
Melezitose	+	+	21/2	P 449/29; P 434/29
Raffinose	+	+	21/2	P 447/09; P 206/06
Rhamnose	+	+	16/7	P 375/05; P 403/11; P 444/21; P 447/09; P 449/03; P 449/26; P 449/29
Ribose	+	+	18/5	P 375/05; P 403/11; P 447/09; P 423/09 ; P 450/03
Sorbitol	–	–	2/21	P 449/26; P 438/12
Starch	–	–	6/17	P 444/21; P 449/26; P 286/08 ; P 416/01 ; P 438/12 ; P 439/06
Xylitol	–	–	1/22	P 206/02
D-Xylose	+	+	22/1	P 206/02
Methyl β-D-xyloside	–	–	5/18	P 375/05; P 444/21; P 421/05 ; P 438/12 ; P 439/06

* Weak hydrolysis of starch, with the exception of strain P 416/01.

† Strains belonging to genotype I are indicated by the use of normal lettering; strains belonging to genotype II are indicated by bold lettering.

KOH string test resulted in a Gram-positive reaction, which was supported by the absence of L-alanine aminopeptidase. No growth was observed on MacConkey agar.

All strains showed positive results for catalase, β-galactosidase, DNase, aesculin and gelatin hydrolysis, as well as for growth in the presence of 2 % NaCl. They assimilated arabinose, gluconate, glucose, malate, maltose, mannitol, mannose, N-acetylglucosamine and produced acid from amygdalin, arbutin, cellobiose, D-fructose, galactose, glycerol, maltose, mannitol, D-mannose, salicin, sucrose and trehalose. The following characteristics were negative for all strains: assimilation of adipate and caprate; acid production from adonitol, D-arabitol, L-arabitol, dulcitol, D-fucose, erythritol, gluconate, 2-ketogluconate, 5-ketogluconate, L-sorbose, D-tagatose and L-xylose; the oxidase reaction; reduction of nitrate to nitrite; indole production; H₂S production from sodium thio-sulphate; urease; arginine dihydrolase and the Voges–Proskauer reaction. Oxidative and fermentative production of acid from glucose, according to the method of Hugh & Leifson (1953) did not occur, but oxidative acid production was positive for all of the strains tested

with API 50CH and for nearly all tested with API 20NE.

Physiological test results demonstrating the differences between the strains studied are given in Table 2. These differences did not correspond to genotype affiliations. Cluster analysis of physiological characteristics showed no clustering according to the genotypes (data not shown). The results of additional physiological tests for representative strains P 333/02^T (I) and P 369/06^T (II) also failed to reveal any discriminatory features. Growth on CNS and TTC media was positive, whereas hydrolysis of cellulose and production of levan from sucrose were negative for both strains. Thus, it was not possible to differentiate effectively between all strains of genotypes I and II on the basis of the morphological or physiological features investigated.

Chemotaxonomic characteristics

Strains P 333/02^T and P 369/06^T were analysed with respect to their menaquinone composition, cellular fatty acid profile, cell-wall sugars and G + C content.

Fig. 1. Phylogenetic tree showing the relationship of the proposed species within the genus *Microbacterium*. The tree is based on a 1422 bp alignment of the 16S rDNA sequences, and was constructed using the neighbour-joining method (Saitou & Nei, 1987). Dots indicate branches of the tree that were also formed using the maximum-likelihood method (Felsenstein, 1981). *Clavibacter michiganensis* was used as an outgroup. The values are the numbers of times that a branch appeared in 100 bootstrap replications. Strains characterized in this study are shown in bold type. The bar indicates the relative sequence divergence (0.01 nucleotide substitutions per site). Sequence data for strains *Microbacterium maritopicum* and *Microbacterium esteraromaticum* were obtained from the Ribosomal Database Project (Larsen *et al.*, 1993).

The menaquinones were fully unsaturated and ranged from MK-9 to MK-13, for P 333/02^T, and from MK-10 to MK-13, for P 369/06^T, respectively. MK-12 and MK-11 were the predominant menaquinones for both strains, constituting more than 70% of the peak area ratio; MK-10 followed at a value of approximately 10%. Strain P 333/02^T contained 56.2% 12-methyl tetradecanoic acid, 17.2% 14-methyl hexadecanoic acid, 13.5% 14-methyl pentadecanoic acid, 7.5% hexadecanoic acid, 3.9% 13-methyl tetradecanoic acid, 1.2% 15-methyl hexadecanoic acid and 0.5% 12-methyl tridecanoic acid. Strain P 369/06^T contained 46.1% 12-methyl tetradecanoic acid, 16.3% 14-methyl pentadecanoic acid, 15.4% 13-methyl tetradecanoic acid, 14.7% 14-methyl hexadecanoic acid, 3.7% 15-methyl hexadecanoic acid, 3.2% hexadecanoic acid and 0.7% 12-methyl tridecanoic acid. The G + C contents of P 333/02^T and P 369/06^T were 67 and 64 mol%, respectively.

The cell-wall peptidoglycan type for P 333/02^T and P 369/06^T was found to be B2β (Schleifer & Kandler, 1972) (L-homoserine)-D-Glu → Gly → D-Orn with glycolyl residues. The cell-wall sugars of strain P 369/06^T were galactose and rhamnose; strain P 333/02^T additionally contained mannose.

Phylogenetic analysis

The 16S rDNA sequencing of P 333/02^T and P 369/06^T gave a similarity level of 99.2%, which represents differences at only 12 of the 1480 bp determined (10 substitutions and 2 additional bases in P 333/02^T). All of the divergent nucleotides of the two sequences were found to be variable among *Microbacterium* spp. Moreover, the different nucleotides of P 333/02^T and P

369/06^T corresponded to those of at least two other *Microbacterium* species. Seven of these differences were located in a variable region that was significantly distinct within the genus *Microbacterium* (positions 74–97; *Escherichia coli* numbering system; Brosius *et al.*, 1978).

To characterize the relationships of the isolates at the species level, the DNA–DNA similarity of strains P 333/02^T and P 369/06^T, as well as that of P 439/06 and P 449/03, representing two pairs of both subgroups, were examined. The results of DNA–DNA reassociation revealed similarities of 41 and 42.7%, suggesting that the strains represent different species. DNA–DNA hybridization between P 333/02^T and the second strain of genotype II (P 439/06) revealed a similarity of 33.6%, supporting the above findings. However, the reassociation between P 369/06^T and P 439/06, both of which are members of genotype II, showed 86.3% similarity, demonstrating that they belong to the same species.

Comparison of 16S rDNA sequences with validly described *Microbacterium* spp. showed that both strains (P 333/02^T and P 369/06^T) evidently belong to the genus *Microbacterium* (Fig. 1). The strains were clustered together using the neighbour-joining method and the maximum-likelihood method, which was supported by high bootstrap values. The best conformities were found with the rRNA sequences of *Microbacterium maritopicum*, *Microbacterium oxydans*, *M. liquefaciens*, *Microbacterium keratanolyticum*, *M. saperdae* and *Microbacterium luteolum*, which were clustered together by both methods. Species displaying similarity values higher than 97% relative to P 333/02^T (genotype I) are listed in Table 3. To clarify the taxonomic position at the species level,

Table 3. Similarity values of 16S rDNA sequences and DNA relatedness between P 333/02^T (= DSM 12966^T) and P 369/06^T (= DSM 13468^T) in comparison to the nearest phylogenetic neighbours of *Microbacterium* spp. and *A. resistens*

Species	16S rDNA similarity to P 333/02 ^T *	DNA reassociation (%) with P 333/02 ^T	16S rDNA similarity to P 369/06 ^T †	DNA reassociation (%) with P 369/06 ^T ‡
P 369/06 ^T	99.20	41.0	—	—
<i>M. aurantiacum</i>	97.00	17.5	97.29	—
<i>M. aurum</i>	97.29	20.6	97.22	—
<i>M. keratanolyticum</i>	98.07	20.6	98.43	33.0
<i>M. kitamiense</i>	97.14	18.3	97.57	—
<i>M. lacticum</i>	97.37	29.7	97.58	—
<i>M. liquefaciens</i>	98.43	28.6	98.29	36.7
<i>M. luteolum</i>	97.86	29.5	97.72	—
<i>M. maritypicum</i>	98.72	29.8	98.57	44.4
<i>M. oxydans</i>	98.65	43.1	98.50	46.2
<i>A. resistens</i>	97.65	21.9	97.79	—
<i>M. saperdae</i>	98.07	37.0	98.00	—
<i>M. terregens</i>	97.15	19.5	97.08	—
<i>M. testaceum</i>	97.65	16.5	98.21	—

* Comparison of 1402 nucleotides and the corresponding sequences.

† Comparison of 1400 nucleotides and the corresponding sequences.

‡ DNA–DNA hybridization against the closest neighbours.

DNA–DNA similarity was examined. All DNA–DNA reassociation values between strain P 333/02^T and the closely related *Microbacterium* spp. were lower than 70 % (Table 3), indicating that the isolate represents a separate species (Wayne *et al.*, 1987).

Hybridization studies performed for the closest phylogenetic neighbours of P 369/06^T (genotype II) showed similar DNA–DNA reassociation values (Table 3), indicating that genotype II represents a new species as well.

DISCUSSION

Restriction analysis of 16S rDNA revealed two different genotypes among the isolates. Comparison of 16S rDNA sequences between representative strains of both subgroups and all validly described species of *Microbacterium* showed a clear affiliation to this genus (Fig. 1). The isolates clustered unambiguously within the monophyletic branch of *Microbacterium*, showing a high level of similarity to the type species *M. lacticum* (DSM 20427^T). The results of chemotaxonomic examinations focusing on selected strains supported these findings. Thus, the tested isolates displayed the major menaquinones MK-12, MK-11 and MK-10, as well as predominating iso- and anteiso-branched fatty acids, as described for the genus *Microbacterium* (Takeuchi & Hatano, 1998a). The G+C content (64 and 67 mol %) is also within the range typical for *Microbacterium* (Takeuchi & Hatano, 1998a). The cell-wall peptidoglycan is of the B2 β type based on D-ornithine (Schleifer & Kandler, 1972). These findings are in accordance with the original description of the genus

Aureobacterium (Collins *et al.*, 1983), accommodated recently in a redefined genus *Microbacterium* (Takeuchi & Hatano, 1998a). Thus, all of the chemotaxonomic features of the isolates correspond to the general phenotypic characteristics of the genus *Microbacterium*.

To clarify the relationship of both genotypes at the species level, DNA–DNA hybridization studies were performed. The results showed reassociation values far below 70 %, the threshold value proposed by Wayne *et al.* (1987) as indicating species status. From these data, it was concluded that both genotypes differentiated by 16S rDNA restriction analysis represent individual species. Comparison of the 16S rDNA sequences of strains representing the genotypes revealed a high level of similarity. Both strains formed a separate branch in the phylogenetic tree, with high bootstrap support (Fig. 1). However, because they demonstrate greater differences in their 16S rDNA sequences than are shown, for example, by the more closely related species *M. oxydans*, *M. liquefaciens* and *M. maritypicum*, 16S rDNA analysis also supported the differentiation of both genotypes at the species level. In contrast to the phylogeny, no physiological feature was found to differentiate all strains of the subgroups. The physiological characteristics of the isolates were similar, although strain-specific differences for several features are demonstrated (Table 2). Clustering on the basis of these divergent features showed no correspondence to the assignment of genotypes (data not shown). Similar results were found for species of the genus *Sulfitobacter* by Pukall *et al.* (1999). *Sulfitobacter mediterraneus* showed the same physiological properties as *Sulfito-*

bacter pontiacus, which can be separated by the restriction patterns of 16S rDNA, DNA–DNA hybridization and fatty acid composition. The similarity of physiological characteristics in association with molecular differences is possibly an expression of an adaptation to the same nutritional compounds for organisms occupying similar ecological niches.

However, differences in the quantitative fatty acid composition (i.e. higher amounts of 13-methyl tetradecanoic acid for strain P 369/06^T) and the additional occurrence of mannose as a cell-wall sugar in strain P 333/02^T are chemotaxonomic features which allowed the differentiation of the proposed type strains.

Analysis of DNA–DNA similarity in comparison to the phylogenetically related *Microbacterium* spp. and *A. resistens* supported the individual species classification within the genus *Microbacterium*, as all re-association values were found to be lower than 70% (Wayne *et al.*, 1987). The two subgroups and the previously described species of the genera *Microbacterium* and *A. resistens* were clearly differentiated by phenotypic characteristics, as shown in Table 4. Almost all species included the closest phylogenetic neighbours (*M. maritimum*, *M. oxydans*, *M. keratanolyticum* and *M. liquefaciens*) and can be easily differentiated by comparison of the composition of the cell wall (sugars and amino acids). *Microbacterium testaceum*, which displayed the same cell wall composition as that of genotype I ('*Microbacterium foliorum*') can be distinguished by growth in the presence of 2% NaCl, H₂S production, and the formation of acid from glucose. *M. luteolum*, showing the same cell wall composition as genotype II ('*Microbacterium phyllosphaerae*'), can be distinguished by the motility of the cells, growth in the presence of NaCl, hydrolysis of gelatin, and H₂S formation. *M. ketosireducens*, the only species with B2β-type peptidoglycan and an unknown cell-wall sugar composition, differed from both genotypes by the motility of the cells, H₂S production, and the assimilation of *N*-acetylglucosamine and malate.

Since both groups of grass-associated isolates can be distinguished from all validly described *Microbacterium* and *Aureobacterium* species on the basis of phenotypic and phylogenetic characteristics, and from each other by analysis of the cell-wall sugars, 16S rDNA restriction analysis using the enzymes *Cfo*I and *Hae*III, and DNA–DNA hybridization, we conclude that both groups deserve a separate species status. Consequently, the names *Microbacterium foliorum* sp. nov. and *Microbacterium phyllosphaerae* sp. nov. are proposed.

Furthermore, the reclassification of *A. resistens* (Funke *et al.* 1998) as *Microbacterium resistens* (Funke *et al.*, 1998) comb. nov. is proposed. *A. resistens* (Funke *et al.* 1998) displayed the general characteristics of the redefined genus *Microbacterium*, and should be reclassified as a result of the taxonomic unification (Takeuchi & Hatano, 1998a).

Description of *Microbacterium foliorum* sp. nov.

Microbacterium foliorum (fo.li.o'rum. L. pl. gen. neut. n. *foliorum* of the leaves).

Cells are Gram-positive, strictly aerobic, non-spore-forming, irregularly shaped rods, which sometimes form V-shapes and are motile by means of a single polar or lateral flagellum. In older cultures, rods are shorter, but a marked rod–coccus cycle does not occur. Colonies are yellow, shiny, slightly convex and round with entire margins. Oxidase, urease, arginine dihydrolase and Voges–Proskauer reactions are negative. Hydrogen sulphide and indole are not produced. Nitrate is not reduced to nitrite. Positive for catalase and β-galactosidase. Gelatin, DNA and aesculin are hydrolysed. Utilization of starch is weak. None of the strains hydrolyses casein and Tween 80, whereas hydrolysis of Tween 60 is strain-dependent. Arabinose, gluconate, glucose, malate, maltose, mannitol, mannose and *N*-acetylglucosamine are assimilated, but caprate and adipate are not used. Citrate and phenylacetate are utilized only by certain strains. Acid production is positive from amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, galactose, glycerol, maltose, mannitol, D-mannose, salicin, sucrose, trehalose and D-xylose, but negative from adonitol, D-arabitol, L-arabitol, dulcitol, D-fucose, erythritol, gluconate, 2-ketogluconate, 5-ketogluconate, inositol, L-sorbose, D-tagatose, xylitol and L-xylose. Acid production is variable between strains of D-arabinose, methyl α-D-glucoside, L-fucose, β-gentiobiose, glycogen, inulin, lactose, methyl α-D-mannoside, melibiose, melezitose, raffinose, rhamnose, ribose, sorbitol, starch and methyl β-D-xyloside. Negative for the fermentative and oxidative production of acid from glucose, according to the method of Hugh & Leifson (1953), but positive for oxidative acid production when tested using API 20NE and API 50CH. Growth occurs in the presence of 2% NaCl. The optimum temperature for growth is approximately 25 °C. At 37 °C, growth is strain-dependent, but growth does not occur at 42 °C. The major menaquinones are MK-12, MK-11 and MK-10. The predominant cellular fatty acids are 12-methyl tetradecanoic acid, 14-methyl hexadecanoic acid and 14-methyl pentadecanoic acid. Contains peptidoglycan of the B2β (L-homoserine)-D-Glu → Gly → D-Orn type, with glycolyl residues. The DNA G + C composition for the type strain is 67 mol %. The cell-wall sugars are galactose, mannose and rhamnose. Isolated from the phyllosphere of grasses and from the litter layer after mulching of the sward. The type strain is DSM 12966^T (= P 333/02^T = LMG 19580^T).

Description of *Microbacterium phyllosphaerae* sp. nov.

Microbacterium phyllosphaerae (phyl.lo.sphae'rae. Gr. n. *phylon* leaf; Gr. fem. n. *sphaira* ball, sphere; M.L. gen. fem. n. *phyllosphaerae* of the phyllosphere).

The morphological and physiological properties are as

Table 4. Differential characteristics of *Microbacterium* spp. and *A. resistens*

Data from this study and Funke *et al.* (1998), Matsuyama *et al.* (1999), Schumann *et al.* (1999), Takeuchi & Yokota (1994), Takeuchi & Hatano (1998a, b), Yokota *et al.* (1993a, b). Abbreviations: +, positive reaction; +w, weakly positive; -, negative; +/-, different results in cited references; d, reaction differs among strains; ND, not determined; y, yellow; yw, yellow white; yb, yellow beige; ly, light yellow; o, orange; GEL, gelatine; starch; H₂S, H₂S production; VP, Voges-Proskauer test; ADH, arginine dihydrolase; ARA, arabinose; NAG, *N*-acetylglucosamine; MLT, malate; CIT, citrate; PAC, phenyl acetate; GLC, glucose; Rha, rhamnose; Gal, galactose; Man, mannose; 6dTal, 6-deoxytalose; Fuc, fucose; Xyl, xylose.

Species	Colour of colony	Motility	Growth		Hydrolysis of		H ₂ S	VP	ADH	Assimilation of :					Acid from GLC	Major Mena-quinone (MK)	Cell wall sugar(s)	Type of Peptidoglycan	Cell wall diamino acid
			37 °C	2% NaCl	GEL	STA				ARA	NAG	CIT	MLT	PAC					
<i>M. foliorum</i> (Genotype I)	y	+	d	+	+	+w	-	-	-	+	+	d	+	d	+	10, 11, 12	Rha, Gal, Man	B2β	Orn
<i>M. phyllosphaerae</i> (Type II)	y	+	d	+	+	+w*	-	-	-	+	+	-	+	d	+†	10, 11, 12	Gal, Rha	B2β	Orn
<i>M. arabinogalactanolyticum</i>	yw/w	-	-	+	+	+	+	+	+	+	+	ND	ND	ND	-	12, 13	Gal	B2β	Orn
<i>M. aurantiacum</i>	o	-	+	+	-	+	+	-	-	d	-	-	d	-	d	12	Rha, Gal, Fuc	B2β	Orn
<i>M. barkeri</i>	w/y	+	+	+	+	+	+	-	+	+	+	+/-	+	-	-	11, 12	Rha, Gal, Glc	B2β	Orn
<i>M. chocolatum</i>	o	-	+	+w	-	+w	+	-	-	-	-	-	-	-	-	12	Rha, Gal, Man [‡] , Xyl [‡]	B2β	Orn
<i>M. esteraromaticum</i>	yw/y	+	d	-	-	+	+	-	-	+	-	-	+/-	-	-	12, 13	Gal, Glc	B2β	Orn
<i>M. flavescens</i>	y	-	-	+	+	+	+	-	-	+	-	-	+/-	-	+	13, 14	Rha, Gal, Glc	B2β	Orn
<i>M. halophilum</i>	yw	-	+	+	+w	+	-	-	-	-	-	-	-	-	+	11, 12, 13	Man, Gal, Glc	B2β	Orn
<i>M. ketosireducens</i>	y	-	-	+	+	+	+	-	-	+	-	d	-	-	+	13	ND	B2β	Orn
<i>M. liquefaciens</i>	y	-	-	+	+	-	+	-	+	-	+	+	+/-	-	-	11, 12	Rha	B2β	Orn
<i>M. luteolum</i>	yw	-	-	-	-	-	+	-	-	+	+	-	+	-	+	12	Rha, Gal	B2β	Orn
<i>M. maritimum</i>	ly	+	+	+	+	-	-	-	-	-	+	+	+	+	+	12	Gal	B2β	Orn
<i>M. oxydans</i>	y	+	+	+	+	-	+	-	-	-	+w	+	+	-	+	11, 12	Rha, Man, Glc, Gal	B2β	Orn
<i>M. saperdae</i>	yw/y	+	-	-	-	+	+	-	+	+	+	-	+	-	+	11, 12	Gal, Glc	B2β	Orn
<i>M. schleiferi</i>	yw/y	-	+w	+	-	-	-	+	-	+w	+w	-	+/-	-	+	11, 12	6dTal, Man, Gal	B2β	Orn
<i>M. terrae</i>	y	-	-	+	+	+	+	-	-	+	-	-	-/w	-	+	13, 14	Rha, Gal, Glc	B2β	Orn
<i>M. terregens</i>	y/yb	-	-	+w	-	-	-	-	-	-	-	-	+	-	+	12, 13	Rha, 6dTal, Gal	B2β	Orn
<i>M. testaceum</i>	o/y-o	+	-	-	+	-	+	-	-	+	+	+/-	+	+w	-	10, 11	Rha, Man, Gal	B2β	Orn
<i>M. thalassium</i>	y	-	-	+	d	+	-	-	-	-	-	-	-	-	+	11, 12	Gal, Glc [§]	B2β	Orn
<i>M. trichothecenolyticum</i>	y	-	-	+w	-	+	+	-	-	-	+	+/-	+	-	+	12, 13	Gal, Glc	B2β	Orn
<i>M. arborescens</i>	o	+/-	-	ND	+	-	+	-	-	+	+	+w	+	-	+	11, 12	6dTal, Man, Gal	B1β	Lys
<i>M. aurum</i>	yw	-	+	+	+	+	+	-	-	-	+w	-	-	-	+	11, 12	Fuc, Gal, Glc	B1β	Lys
<i>M. imperiale</i>	o	+	+	ND	-	+	+/-	-	-	+	+w	-	+	-	+	11, 12	Rha, Man, Gal	B1β	Lys
<i>M. dextranolyticum</i>	w	-	-	ND	-	-	+	+	-	+	-	-	+	+	+	11, 12	6dTal, Man, Gal	B1α	Lys
<i>M. hominis</i>	yw	-	+	+	-	-	+	+	-	+	+	+	+	-	+	11, 12	Rha, 6dTal, Gal, Man	B1α	Lys
<i>M. lacticum</i>	yw/y	-	-	+w	-	+	-	-	-	+w	+	-	+	-	+	11, 12	Rha, Man, Gal	B1α	Lys
<i>M. laevaniformans</i>	yw/y	-	+	+	-	+	+	+	d	-	-	d	+	-	+	11, 12	Rha, Man, Gal, Xyl	B1α	Lys
<i>M. keratanolyticum</i>	y	+	-	+	+	-	+	-	+	+w	+	-	-	-	-	12, 13	Gal	B2α	Orn
<i>A. resistens</i>	y	-	+	+	+	-	-	-	-	-	+	+	-	-	+	ND	ND	B2α	Orn
<i>M. kitamiense</i>	o	-	+	+	+	+	-	-	-	-	-	-	-	-	+	11, 12	ND	ND	Orn

* The majority of strains showed a weak reaction with the exceptions of one strain with strong hydrolysis and one without hydrolysis.

† Determined by API 20NE; one tested strain was not able to produce acid.

‡ Traces.

§ Depending on strain; Gal, Glc, Rha, Man.

described for *M. foliorum*, but the hydrolysis of Tween 80, casein and starch varies between strains. Assimilation of citrate is negative. Acid production from β -gentiobiose, as well as from rhamnose, is positive for all strains. Acid production is strain-dependent for L-arabinose, inositol, xylitol and D-xylose. Oxidative acid production from glucose, tested with API 20NE, is positive for almost all strains. The major menaquinones are MK-12, MK-11 and MK-10. The predominant cellular fatty acids are 12-methyl tetradecanoic acid, 14-methyl pentadecanoic acid, 13-methyl tetradecanoic acid and 14-methyl hexadecanoic acid. The DNA G + C composition for the type strain is 64 mol%. The peptidoglycan is of the B2 β (L-homoserine)-D-Glu $\rightarrow$ Gly $\rightarrow$ D-Orn type, with glycolyl residues. The cell-wall sugars are galactose and rhamnose. Isolated from the phyllosphere of grasses and from the litter layer after mulching of the sward. The type strain is DSM 13468^T (= P 369/06^T = LMG 19581^T).

Description of *Microbacterium resistens* (Funke et al. 1998) comb. nov., basonym *Aureobacterium resistens*

The morphological, cultural and physiological properties were reported by Funke *et al.* (1998). In addition, the major menaquinones are MK-12 and MK-13, the arginine dihydrolase and Voges-Proskauer reactions are negative, and growth in the presence of 2% NaCl is positive. The type strain, isolated from human clinical specimens, is DSM 11986^T (= CCUG 38312^T = DMMZ 1710^T).

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REFERENCES

Behrendt, U., Müller, T. & Seyfarth, W. (1997). The influence of extensification in grassland management on the populations of micro-organisms in the phyllosphere of grasses. *Microbiol Res* **152**, 75–85.

Behrendt, U., Ulrich, A., Schumann, P., Erler, W., Burghardt, J. & Seyfarth, W. (1999). A taxonomic study of bacteria isolated from grasses: a proposed new species *Pseudomonas graminis* sp. nov. *Int J Syst Bacteriol* **49**, 297–308.

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 USA* **75**, 4801–4805.

Collins, M. D. & Bradbury, J. F. (1992). The genera *Agromyces*, *Aureobacterium*, *Clavibacter*, *Curtobacterium*, and *Microbacterium*. In *The Prokaryotes*, pp. 1355–1368. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin: Springer.

Collins, M. D., Jones, D., Keddle, R. M., Kroppenstedt, R. M. &

Schleifer, K. H. (1983). Classification of some coryneform bacteria in a new genus *Aureobacterium*. *Syst Appl Microbiol* **4**, 236–252.

Felsenstein, J. (1981). Evolutionary tree from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, WA, USA.

Funke, G., Lawson, P. A., Nolte, F. S., Weiss, N. & Collins, M. D. (1998). *Aureobacterium resistens* sp. nov., exhibiting vancomycin resistance and teicoplanin susceptibility. *FEMS Microbiol Lett* **158**, 89–93.

Groth, I., Schumann, P., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Beutenbergia cavernae* gen. nov., sp. nov., an L-lysine-containing actinomycete isolated from a cave. *Int J Syst Bacteriol* **49**, 1733–1740.

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.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Komagata, K. & Suzuki, K.-I. (1987). Lipid and cell-wall analysis in bacterial systematics. In *Methods in Microbiology*, vol. 19, pp. 161–207. Edited by R. R. Colwell & R. Grigorova. London: Academic Press.

Larsen, N., Olsen, G. J., Maidak, B. L., McCaughey, M. J., Overbreek, R., Macke, T. J., Marsh, T. L. & Woese, C. R. (1993). The ribosomal database project. *Nucleic Acids Res* **21**, 3021–3023.

Legard, D. E., McQuilken, M. P., Whipps, J. M., Fenlon, J. S., Fermor, T. R., Thompson, I. P., Bailey, M. J. & Lynch, J. M. (1994). Studies of seasonal changes in the microbial populations on the phyllosphere of spring wheat as a prelude to the release of a genetically modified microorganism. *Agric Ecosyst Environ* **50**, 87–101.

McInroy, J. A. & Kloepper, J. W. (1995). Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil* **173**, 337–342.

Martin, K., Schumann, P., Rainey, F. A., Schuetze, B. & Groth, I. (1997). *Janibacter limosus* gen. nov., a new actinomycete with meso-diaminopimelic acid in the cell wall. *Int J Syst Bacteriol* **47**, 529–534.

Matsuyama, H., Kawasaki, K., Yumoto, I. & Shida, O. (1999). *Microbacterium kitamiense* sp. nov., a new polysaccharide-producing bacterium isolated from the wastewater of a sugar-beet factory. *Int J Syst Bacteriol* **49**, 1353–1357.

Pukall, R., Buntetu, D., Frühling, A., Rohde, M., Kroppenstedt, R. M., Burghardt, J., Lebaron, P., Bernard, L. & Stackebrandt, E. (1999). *Sulfitobacter mediterraneus* sp. nov., a new sulfite-oxidizing member of the α -Proteobacteria. *Int J Syst Bacteriol* **49**, 513–519.

Rudolph, K. & Marvidis, A. (1990). Flagella, motility, chemotaxis and helicity. In *Methods in Phytobacteriology*, pp. 441–444. Edited by Z. Klement, K. Rudolph & D. C. Sands. Budapest: Akademiai Kiado.

Ryu, E. (1938). On the Gram-differentiation of bacteria by the simplest method. *J Jpn Soc Vet Sci* **17**, 58–63.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sands, D. C. (1990). Physiological criteria – determinative tests. In *Methods in Phytobacteriology*, pp. 133–143. Edited by Z.

- Klement, K. Rudolph & D. C. Sands. Budapest: Akademiai Kiado.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schumann, P., Rainey, F. A., Burghardt, J., Stackebrandt, E. & Weiss, N. (1999). Reclassification of *Brevibacterium oxydans* (Chatelain and Second 1966) as *Microbacterium oxydans* comb. nov. *Int J Syst Bacteriol* **49**, 175–177.
- Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy*. San Francisco: W. H. Freeman.
- 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.
- Süßmuth, R., Eberspächer, J., Haag, R. & Springer, W. (1987). *Biochemisch-mikrobiologisches Praktikum*. Stuttgart: Thieme.
- Takeuchi, M. & Hatano, K. (1998a). Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a redefined genus *Microbacterium*. *Int J Syst Bacteriol* **48**, 739–747.
- Takeuchi, M. & Hatano, K. (1998b). Proposal of six new species in the genus *Microbacterium* and transfer of *Flavobacterium marinotipicum* ZoBell and Upham to the genus *Microbacterium* as *Microbacterium maritipicum* comb. nov. *Int J Syst Bacteriol* **48**, 973–982.
- Takeuchi, M. & Yokota, A. (1994). Phylogenetic analysis of the genus *Microbacterium* based on 16S rRNA gene sequences. *FEMS Microbiol Lett* **124**, 11–16.
- Thompson I. P., Bailey M. J., Fenlon J. S. & 8 other authors (1993). Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (*Beta vulgaris*). *Plant Soil* **150**, 177–191.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- 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.
- Vidaver, A. K. & Davis, M. J. (1994). Coryneform plant pathogens. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 2nd edn, pp. 104–113. Edited by N. W. Schaad. St Paul, MN: American Phytopathological Society Press.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Yokota, A., Takeuchi, M., Sakane, T. & Weiss, N. (1993a). 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.
- Yokota, A., Takeuchi, M. & Weiss, N. (1993b). Proposal of two new species in the genus *Microbacterium*: *Microbacterium dextranolyticum* sp. nov. and *Microbacterium aurum* sp. nov. *Int J Syst Bacteriol* **43**, 549–554.