Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a redefined genus *Microbacterium*

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The 16S rRNA gene sequences of 19 strains, 11 strains representing validated *Aureobacterium* or *Microbacterium* species and eight strains of non-valid species or isolates, were determined. These sequences were aligned with the sequences of other validated *Aureobacterium* and *Microbacterium* species and related actinobacteria. A comparative sequence analysis of 43 strains revealed that the species of the genera *Aureobacterium* and *Microbacterium* form a monophyletic association in which species of both genera are intermixed. The high similarity in phylogenetic properties found in the species within both genera and the close relationship in physiological and chemotaxonomic features other than the diamino acid in the cell wall, provided strong evidence that the genera *Aureobacterium* and *Microbacterium* should be unified. An emended genus *Microbacterium* is proposed for the two combined genera. The following validated *Aureobacterium* species were combined to the genus *Microbacterium*: *Aureobacterium arabinogalactanolyticum* to *Microbacterium arabinogalactanolyticum*, *Aureobacterium barkeri* to *Microbacterium barkeri*, *Aureobacterium esteraromaticum* to *Microbacterium esteraromaticum*, *Aureobacterium flavescens* to *Microbacterium flavescens*, *Aureobacterium keratanolyticum* to *Microbacterium keratanolyticum*, *Aureobacterium liquefaciens* to *Microbacterium liquefaciens*, *Aureobacterium luteolum* to *Microbacterium luteolum*, *Aureobacterium saperdae* to *Microbacterium saperdae*, *Aureobacterium schleiferi* to *Microbacterium schleiferi*, *Aureobacterium terrae* to *Microbacterium terrae*, *Aureobacterium terregens* to *Microbacterium terregens*, *Aureobacterium testaceum* to *Microbacterium testaceum*, and *Aureobacterium trichothecenolyticum* to *Microbacterium trichothecenolyticum*.

Keywords: *Microbacterium*, *Aureobacterium*, 16S rDNA analysis

INTRODUCTION

On the basis of 5S ribosomal RNA (rRNA) sequencing and associated chemotaxonomic data, Park *et al.* proposed the family *Microbacteriaceae* (25) to accommodate Gram-positive bacteria with G+C-rich DNA and with the rare group-B-type peptidoglycan (30); and the genera *Agromyces* (11), *Aureobacterium* (5), *Clavibacter* (8), *Curtobacterium* (42) and *Microbacterium* (24) were accommodated in this family.

Recently, Stackebrandt *et al.* (34) proposed a new hierarchic classification system in which phylogenetically neighbouring taxa at the genus level are clustered into families, suborders, subclasses and a class, based on 16S ribosomal DNA (rDNA)/rRNA sequence-based phylogenetic clustering and the presence of taxon-specific 16S rDNA/RNA signature nucleotides. They proposed the family *Microbacteriaceae* to accommodate the type genus *Microbacterium* as well as the genera *Agrococcus* (12),

The DDBJ accession numbers for the 16S rDNA sequences determined in this study are AB004713–AB004728 (Table 1).

Table 1. Bacterial strains used and their 16S rDNA accession numbers

Species	Strain (IFO no.)	Other designation*	Source	DDBJ accession no.
Aureobacterium arabinogalactanolyticum	14344^{T}			AB004715
Aureobacterium flavescens	15039^{T}	ATCC 13348^{T}		AB004716
Aureobacterium keratanolyticum	13309^{T}			AB004717
Aureobacterium luteolum	15074^{T}	DSM 20143^{T}		AB004718
Aureobacterium saperdae	15038^{T}	ATCC 19272^{T}		AB004719
Aureobacterium terrae	15300^{T}			AB004720
Aureobacterium terregens	12961^{T}	ATCC 13345^{T}		AB004721
Aureobacterium trichothecenolyticum	15077^{T}	JCM 1358^{T}		AB004722
Aureobacterium schleiferi	15075^{T}	DSM 20489^{T}		AB004723
‘*Aureobacterium ketoreductum*’	14548			AB004724
‘*Aureobacterium ketoreductum*’	14549			
‘*Chromobacterium chocolatum*’	3758	BUCSAV 207, NCIB 8181		AB004725
Flavobacterium marinotypicum†	15779	ATCC 19260, NCMB 1050		
Microbacterium laevaniformans	15234	NCFB 2288, ATCC 49090		AB004726
Microbacterium laevaniformans	15235	NCFB 2289, ATCC 49091		
Microbacterium sp.	15708	CDC group A-4	Lung aspirate	AB004727
Microbacterium sp.	15709	CDC group A-5	Blood	AB004728
Strain no. 10	16060		Mangrove rhizosphere	AB004713
Strain no. 71	16061		Mangrove rhizosphere	
Strain no. 76	16062		Mangrove rhizosphere	AB004714

* ATCC, American Type Culture Collection, Rockville, MD, USA; BUCSAV, Institute of Biology, Czechoslovak Academy of Sciences, Prague, Czech Republic; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; JCM, Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Japan; NCFB, National Collection of Food Bacteria, Reading, UK; NCMB(NCIB), National Collection of Industrial and Marine Bacteria, Aberdeen, UK.

† Sequence data for ATCC 19260^{T} was obtained from the Ribosomal Database Project.

Agromyces, *Aureobacterium*, *Clavibacter*, *Curtobacterium* and *Rathayibacter* (45). Most recently, the new genera *Leucobacter* (39) and *Cryobacterium* (36) were proposed. Phylogenetic analyses based on 16S rDNA sequences revealed that these two genera are also positioned at separate branches in the family *Microbacteriaceae*. At present, nine genera are included in this family.

The genus *Microbacterium* was proposed by Orla-Jensen in 1919 (24), and its description was emended by Collins *et al*. in 1983 (4). Six species have been described previously (43). Recently, the CDC coryneform group A-4 and A-5 bacteria (15) have been reported to belong to the genus *Microbacterium* (10). On the other hand, the genus *Aureobacterium* was proposed by Collins *et al*. in 1983 (5), and 13 species have been described previously (44). It is, however, now evident that the organisms of both the genera *Microbacterium* and *Aureobacterium* are phylogenetically intermixed and form a monophyletic group (26, 38), although they are separate taxa based primarily on cell wall differences: the presence of an L-diamino acid, L-lysine, in the cell wall (B1 type of peptidoglycan) is characteristic of the species in the genus *Microbacterium* (29, 30), and the presence of a D-diamino acid, D-ornithine, in the cell wall (B2-type peptidoglycan) is characteristic of the species in the genus *Aureobacterium* (44). The other chemotaxonomic and phenotypic properties of the organisms of both the genera *Microbacterium* and *Aureobacterium* exhibit very similar profiles; the organisms of these genera are characterized by the presence of *N*-glycolyl residues in the cell walls, by having major isoprenoid quinones MK-11, MK-12 and MK-13 and/or minor isoprenoid menaquinones MK-10 or MK-14, and by G + C contents of 65–76 mol%.

To examine the phylogenetic relationships within and between the genera *Microbacterium* and *Aureobacterium*, we determined the almost complete 16S rRNA gene sequences of 19 strains as shown in Table 1, and compared them with published sequences of species included in the genera *Microbacterium* and *Aureobacterium* and with other members of the family *Microbacteriaceae*.

On the basis of this study, we propose the union of the genera *Microbacterium* and *Aureobacterium* in a redefined genus *Microbacterium*.

METHODS

Bacterial strains and culture conditions. Strains used are listed in Table 1. The validated species *Flavobacterium marinotypicum* (1) appears on the Approved Lists of Bacterial Names (31), but it is now excluded from the genus

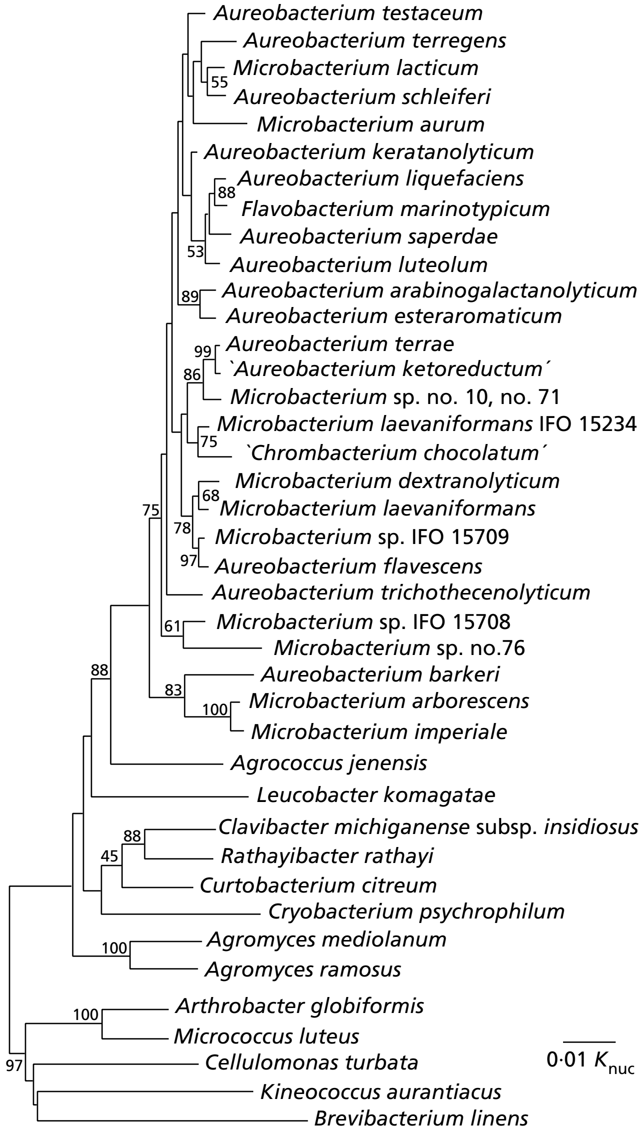

Fig. 1. Phylogenetic dendrogram based on a comparison of the 16S rDNA sequences of *Microbacterium* and *Aureobacterium* species and some close relatives. The numerals indicate the percentages of bootstrap samplings, derived from 1000 samples, supporting the internal branches (9). Bar, 0·01 K_{nuc} unit.

Flavobacterium because of its Gram-positive reaction and its motility (16). Strain IFO 14548 and strain IFO 14549 were identified as '*Aureobacterium ketoreductum*' by K. Imai in 1986, since they reduce 2,7-anhydro-β-D-arabino-2,5-heptodiulopyranose to 2,7-anhydro-β-D-ido-heptulopyranose (unpublished data), and '*Chromobacterium chocolatum*' was isolated by M. H. Knutsen in 1944 (2). However, the names of these strains do not appear on the Approved Lists of Bacterial Names (31). Two strains of *Microbacterium laevaniformans*, IFO 15234 and IFO 15235 had been assigned to the genus *Aureobacterium* based on their chemotaxonomic features (43). *Microbacterium* sp. strains IFOP 15708 and IFO 15709 were isolated from clinical specimens (10). Strains no. 10 (= IFO 16060), no. 71 (= IFO 16061) and no. 76 (= IFO 16062) were isolated from soil in mangrove rhizospheres in Okinawa prefecture. Each strain was cultured at 28 °C with aerobic shaking in a peptone-yeast extract medium, which contained 1 % (w/v) peptone, 0·2 % yeast extract, 0·2 % NaCl and 0·2 % D-glucose (pH 7·2). Cells used for biochemical tests were harvested by centrifugation during the stationary phase, washed with water, and lyophilized.

Chemical analyses. Cell walls were prepared from ca. 500 mg (dry wt) bacterial cells as described by Schleifer & Kandler (30). Amino acids in the acid hydrolysate of the cell walls were identified by two-dimensional TLC (13), and assayed with a model LC-6AD HPLC apparatus as described previously (41, 43). Cellular fatty acids were extracted from dried cells (50 mg) by acid methanolysis, purified (43), and analysed by GLC-MS with a GCMS-QP5000 spectrometer (Shimadzu) combined with a CLASS-5000 MS Workstation computer system. GLC analyses were performed with a GC-17A gas chromatograph (Shimadzu). A BPX70 capillary column (SGE) containing 70 % cyanopropyl equivalent modified siloxane (50 m × 0·25 nm) was used at 80 °C for 2 min, 80–150 °C at 15 °C min^{-1}, 150–250 °C at 8 °C min^{-1}, and then 250 °C for 5 min, with helium as carrier gas at a flow rate of 1·4 ml min^{-1}. Menaquinones were extracted from dry cells (200 mg) with chloroform/methanol (2:1, v/v), purified by silica gel TLC (Kieselgel $60F_{254}$; Merck) using hexane/diethyl ether (85:15, v/v) as the solvent, extracted with diethyl ether, dried under nitrogen stream, and then analysed by HPLC by using a Shimadzu model LC-5A instrument equipped with a Zorbax octyldecyl silane column (4·6 × 150 mm). Glycolate tests were performed as described by Uchida & Aida (40).

16S rDNA sequence determination and phylogenetic analysis. 16S rRNA-specific DNA was amplified by PCR and sequenced directly (14) with a Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Life Science) and a model Pharmacia ALF DNA sequencer (Pharmacia LKB) following the manufacturers' protocols. The sequences were aligned to published sequences from DDBJ, GenBank and EMBL. Nucleotide substitution rates (K_{nuc} values) were calculated (18), and the phylogenetic tree was constructed by the neighbour-joining method (27). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (9) with 1000 replicates.

Nucleotide sequence accession numbers. The accession numbers of the 16S rDNA sequences used for comparison with the sequences which we determined are as follows: *Agrococcus jenensis* DSM 9580^T, X92492; *Agromyces cerinus* subsp. *cerinus* JCM 9083^T, D45060; *Agromyces fucosus* subsp. *hippuratus* JCM 9086^T, D45061; *Agromyces mediolanus* JCM 3346^T, X77449; *Agromyces ramosus* JCM 3108^T, X77447; *Arthrobacter globiformis* DSM 20124^T, M23411; *Aureobacterium barkeri* DSM 20145^T, X77446; *Aureobacterium dextranolyticum* IFO 14592^T, AB007417; *Aureobacterium liquefaciens* DSM 20638^T, X77444; *Aureobacterium testaceum* DSM 20166^T, X77445; *Brevibacterium linens* DSM 20425^T, X77451; *Cellulomonas turbata* DSM 20577^T, X83806; *Clavibacter michiganensis* subsp. *insidiosus* JCM 1369^T, D45051; *Clavibacter michiganensis* subsp. *michiganensis* JCM 3345^T, D45059; *Clavibacter michiganensis* subsp. *nebraskense* DSM 7483^T, X77434; *Clavibacter xyli* subsp. *cynodontis* ATCC 33973^T, M60935; *Cryobacterium psychrophilum* JCM 1463^T, D56058; *Curtobacterium citreum* DSM 20528^T, X77436; *Curtobacterium* *luteum* DSM 20542^T, X77437; *Kineococcus aurantiacus* IFO 15268^T, AB007420; *Leucobacter komagatae* IFO 15245^T, AB007419; *Microbacterium arborescens* IFO 3750^T, AB007421; *Microbacterium aurum* IFO 15204^T, AB007418; *Microbacterium imperiale* IFO 12610^T, AB007414; *Microbacterium lacticum* IFO 14135^T, AB7415; *Microbacterium laevaniformans* IFO 14471^T, AB007416; *Micrococcus luteus* DSM 20030^T, M38242; *Rathayibacter rathayi* DSM 7458^T, X77439; and *Rathayibacter tritici* DSM 7486^T, X77438.

RESULTS

Phylogenetic analysis

Almost complete 16S rDNA sequences of 19 strains were determined, ranging in length from 1410 to 1533 nucleotides. They were compared with the sequences of 11 strains of the genera *Aureobacterium* and *Microbacterium*, and with those of 13 strains of related actinobacteria. The sequence similarity calculations were based on 1201 nucleotides, because only partial sequences were available for some of the reference strains. '*A. ketoreductum*' strains IFO 14548 and IFO 14549, *M. laevaniformans* strains IFO 15234 and IFO 15235, and strains no. 10 and no. 71 had identical 16S rDNA sequences, respectively. The phylogenetic dendrogram in Fig. 1 clearly shows that all strains of the genera *Microbacterium* and *Aureobacterium* from one large cluster in which species of both genera are intermixed. The levels of sequence similarity found between species of both genera *Microbacterium* and *Aureobacterium* ranged from 96·8 to 99·7%, those between species of other members of the family *Microbacteriaceae*, the genera *Agrococcus*, *Agromyces*, *Clavibacter*, *Cryobacterium*, *Curtobacterium*, *Leucobacter* and *Rathayibacter* ranged from 93·4 to 96·9%, and those between species of other genera which were not contained in the family *Microbacteriaceae* ranged from 90·9 to 94·1%, respectively (data not shown). The high sequence similarity between species of both genera *Microbacterium* and *Aureobacterium* indicates the phylogenetic coherence of the two genera.

A search for nucleotide positions at which the nucleotides are the same for all or most of the strains belonging to the *Microbacterium–Aureobacterium* cluster resulted in a set of signatures which support the phylogenetic placement of this cluster. As shown in Table 2, the pattern of 16S rDNA signatures consists of nucleotides G at position 232 (*Escherichia coli* numbering), U at position 279, A at position 780, U-A base pair at positions 838–856, and A-U base pair at positions 929–1386, which are unique for all the strains of *Microbacterium–Aureobacterium* studied. All strains belonging to the *Microbacterium–Aureobacterium* cluster and one strain of the genus *Agrococcus* are characterized by a unique guanosine at position 232 and adenosine at position 780, although all strains of the other genera have adenosine and guanosine or cytidine at these positions. These results correspond with the phylogenetic profile of *Agrococcus jenensis* (Fig. 1), which formed a branch at the base of the *Microbacterium–Aureobacterium* cluster, as mentioned previously (12). This set of signature nucleotides, as well as the low levels of 16S rDNA sequence similarity to the other taxa, supports the distinctness of the *Microbacterium–Aureobacterium* lineage, which should be described at the level of a single genus.

Chemotaxonomic characteristics

The chemotaxonomic characteristics of 11 strains determined in this study and those of 19 strains

Table 2. 16S rRNA signature nucleotide positions that distinguish the *Microbacterium–Aureobacterium* lineage from other members of the family *Microbacteriaceae*

Genus (no. of strains compared)	Position									
	69–99	129	232	279	443–491	502–543	770–809	780	838–856	929–1386
Microbacterium (29)	G/A*-U	C/U†	G	U	C/U‡-G	G/A§-C/U‖	G-C/G¶	A	U-A	A-U
Agrococcus (1)	A-U	C	G	A	C-G	A-U	G-G	A	A-U	G-C
Agromyces (4)	A-U	U	A	U	U-G	A-U	C-G	G	A-U	G/A-C
Clavibacter (4)	G-U	U	A	A	U-G	A-U	C-G	C	A-U	G-C
Cryobacterium (1)	G-U	C	A	A	U-G	A-U	C-N	G	A-U	G-C
Curtobacterium (2)	A-U	U	A	A	U-G	A-U	C-G	G	A-U	G-C
Leucobacter (1)	A-A	U	A	A	U-G	A-U	C-G	G	A-U	G-C
Rathayibacter (2)	A-U	U	A	A	U-G	A-U	C-G	G	A-U	G-C

* *Aureobacterium barkeri* and *Aureobacterium esteraromaticum* have adenosine.

† *Aureobacterium aurum* has uridine.

‡ *Aureobacterium keratanolyticum* has uridine.

§ *Aureobacterium terregens* has adenosine.

‖ *Aureobacterium arabinogalactanolyticum*, *Aureobacterium aurum*, *Aureobacterium esteraromaticum*, *Aureobacterium terregens* and *Microbacterium imperiale* have uridine.

¶ *Aureobacterium liquefaciens* has guanosine.

Table 3. Chemotaxonomic characteristics of the species of the genera *Microbacterium* and *Aureobacterium*

Data from this study (*) and previous studies (43, 44).

Species	Strain	G+C content (mol%)	Menaquinones (MK)	Major cellular fatty acids	Amino acids in the cell-wall	position 3 linkage
A. arabinogalactanolyticum	14344ᵀ	69·3	12, 13	ai15, ai17, i16	L-Hsr	D-Orn
A. barkeri	15036ᵀ	68·7	11, 12	ai15, ai17, i16	L-Hsr	D-Orn
A. esteraromaticum	3751ᵀ	68·8	12, 13	ai15, ai17, i16	L-Hsr	D-Orn
A. flavescens	15039ᵀ	66·9	13, 14	ai15, ai17, i16	L-Hsr	D-Orn
A. keratanolyticum	13309ᵀ	66·5	12, 13	ai15, ai17, i16	L-Orn	D-Orn
A. liquefaciens	15037ᵀ	68·6	11, 12	ai15, ai17, i16	L-Hsr	D-Orn
A. luteolum	15074ᵀ	70·6	12	ai15, ai17, i16	L-Hsr	D-Orn
A. saperdae	15038ᵀ	69·1	11, 12	ai15, ai17, i16	L-Hsr	D-Orn
A. terrae	15300ᵀ	70·7	13, 14	ai15, ai17, i16	L-Hsr	D-Orn
A. terregens	12961ᵀ	68·6	12, 13	ai15, ai17, i16	L-Hsr	D-Orn
A. testaceum	12675ᵀ	67·7	10, 11	ai15, ai17, i16	L-Hsr	D-Orn
A. trichothecenolyticum	15077ᵀ	69·0	12, 13	ai15, ai17, i16	L-Hsr	D-Orn
A. schleiferi	15075ᵀ	66·9	10, 11, 12	ai15, ai17, i16	L-Hsr	D-Orn + D-Hyo
'*A. ketoreductum*'*	14548	69·8	13	ai15, ai17, i16	Hsr	Orn
'*A. ketoreductum*'*	14549	69·7	13	ai15, ai17, i16	Hsr	Orn
'*C. chocolatum*'*	3758	69·5	12	ai15, ai17, i16	Hsr	Orn
*F. marinotypicum**	15779	71·6	12	ai15, (ai17), i16	Hsr	Orn
M. arborescens	3750ᵀ	71·0	11, 12	ai15, ai17, i16	L-Hsr	L-Lys
M. aurum	15204ᵀ	69·2	11, 12	ai15, ai17, i16	L-Hsr	L-Lys
M. dextranolyticum	14592ᵀ	68·3	11, 12	ai15, ai17, i16	L-Lys	L-Lys
M. imperiale	12610ᵀ	71·2	11, 12	ai15, ai17, i16	L-Hsr	L-Lys
M. lacticum	14135ᵀ	70·0	11, 12	ai15, ai17, i16	L-Lys	L-Lys
M. laevaniformans	14471ᵀ	70·5	11, 12	ai15, ai17, i16	L-Lys	L-Lys
*M. laevaniformans**	15234	70·3	12	ai15, ai17, i16	L-Hsr	D-Orn
*M. laevaniformans**	15235	70·1	12	ai15, ai17, i16	L-Hsr	D-Orn
Microbacterium sp.*	15708	71·2	11, 12	ai15, ai17, i16	Lys	Lys
Microbacterium sp.*	15709	71·5	11, 12	ai15, ai17, i16	Lys	Lys
Strain no. 10*	16060	69·7	11, 12	ai15, ai17, i16	Hsr	Orn
Strain no. 71*	16061	69·1	11, 12	ai15, ai17, i15, i16	Hsr	Orn
Strain no. 76*	16062	67·2	11, 12, 13	ai15, ai17, i15, i16	Hsr	Orn

ai, anteiso-branched acid; i, iso-branched acid; L-Hsr, L-homoserine; D-Orn, D-ornithine; D-Hyo, D-hydroxyornithine; L-Lys, L-lysine.

previously studied are summarized in Table 3. The diamino acid in the cell wall of '*Aureobacterium ketoreductum*' strains IFO 14548 and IFO 14549, '*Chromobacterium chocolatum*' strain IFO 3758, *F. marinotypicum* strain IFO 15779, and strains no. 10, no. 71 and no. 76 was ornithine, and that of *Microbacterium* sp. strains IFO 15708 and IFO 15709 was lysine. The molar ratio of ornithine, alanine, glutamic acid (plus hydroxyglutamic acid) and glycine of cell wall hydrolysates of '*Aureobacterium ketoreductum*' strains IFO 14548 and IFO 14549 were 0·82:1·00:0·81:1·65, those of '*Chromobacterium chocolatum*' strain IFO 3758 were 1·10:1·00:0·64:4·86, those of *F. marinotypicum* IFO 15779 were 1·10:1·00:1·04:3·06, and those of strains no. 10, no. 71 and no. 76 were 0·76 ∼ 1·10:1·00:1·03 ∼ 1·20:2·91 ∼ 3·60. Homoserine was detected in all strains mentioned above, but not calculated exactly. The amino acids in the cell-wall hydrolysates of *Microbacterium* sp. strains IFO 15708 and IFO 15709 were lysine, alanine, glutamic acid (plus hydroxyglutamic acid) and glycine in the molar ratio of 1·87:1·00:1·02:2·80 and 2·29:1·00:1·26:3·47, respectively. Although the configurations of these amino acids were not determined, the peptidoglycan types are suggested to be B2β for the former strains and B1α for the latter. The G+C contents of the DNAs of these strains ranged from 67·2 to 71·6 mol%. Cellular fatty acids were composed mainly of anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$. The major menaquinone of the two strains of '*Aureobacterium ketoreductum*' was MK-13, that of '*Chromobacterium chocolatum*' and *F. marinotypicum* was MK-12, and those of strain nos 10 and 71 and *Microbacterium* sp. strains IFO 15708 and IFO 15709 were MK-11 and MK-12 and those of strain no. 76 were MK-11, MK-12 and MK-13.

DISCUSSION

Most actinomycete genera are homogeneous with respect to their chemotaxonomic features (20, 32), and the differences in the diamino acids in the third position of the tetrapeptide of the peptidoglycan are one of the most important chemotaxonomical markers (7).

However, there are a few exceptions in some actinobacteria, for instance, the genera *Propionibacterium* (6), *Bifidobacterium* (28) and *Cellulomonas* (33, 35). In the case of the genus *Propionibacterium*, *Propionibacterium freudenreichii* contains *meso*-diaminopimelic acid, but all the other species belonging to the genus *Propionibacterium* contain LL-diaminopimelic acid. On the other hand, the taxonomic positions of the genera *Cellulomonas* and *Oerskovia* have long been controversial. An emended genus *Cellulomonas* was proposed by Stackebrandt *et al.* (35) in 1982 for the two genera *Cellulomonas* and *Oerskovia* because of the close natural relationship and the high similarity in physiological, biochemical and metabolic properties found between species of the two genera, though current opinion based primarily on cell wall differences was that these genera should retain their separate taxa (33). It is however, now evident that species of both genera are phylogenetically intermixed and form a monophyletic group in spite of the differences in cell-wall diamino acids in the organisms of both genera. In some species of the genus *Bifidobacterium*, L-lysine and L-ornithine are both present in varying amounts in the same peptidoglycan (17, 28), and different murein types are found in one strain. Thus, in some species of particular genera, there is not a good correlation between the type of diamino acids in their peptidoglycan and the phylogenetic trees.

In the case of the organisms of genera *Microbacterium* (43) and *Aureobacterium* (44), it had been reported that they exhibit very similar profiles in phenotypic and chemotaxonomic properties except for the diamino acids in the cell walls, and that the species of the two genera show high similarity in phylogenetic properties.

In this paper, we determined the almost complete 16S rRNA gene sequences of 19 strains, containing 11 strains representing validated *Aureobacterium* and *Microbacterium* species, eight strains of non-valid species and isolates, and compared them with the sequences of other validated *Aureobacterium* and *Microbacterium* species and related actinobacteria, to clarify the phylogenetic relationships within and between the genera *Microbacterium* and *Aureobacterium*.

A comparative sequence analysis of 43 strains revealed that all strains formed a monophyletic cluster (sequence similarity $>96{\cdot}8\%$), in which they were intermixed with each other and did not form clusters according to their taxonomic affiliation. These results show that phylogenetic relatedness found in the genera *Microbacterium* and *Aureobacterium* does not reflect the differences in the configuration of diamino acids in the interpeptide bridge (peptidoglycan type B1 or B2) or in the amino acid at position 3 of the peptide unit in the peptidoglycan (peptidoglycan type $B1\alpha/B1\beta$ or $B2\alpha/B2\beta$).

Comparative analysis of the 16S rDNA also allowed us to identify a set of signature nucleotides that is considered to characterize the *Microbacterium–Aureobacterium* lineage, and the signature as given in Table 2 is true of all *Microbacterium* and *Aureobacterium* strains that were studied. This set of signature nucleotides supports the distinctness of the *Microbacterium–Aureobacterium* lineage, which should be described at the level of a single genus.

This conclusion is also supported by phenotypic characteristics, e.g. chemotaxonomic profiles and morphological properties (Table 4).

Therefore, we propose the union of the genera *Microbacterium* and *Aureobacterium*, and the name *Microbacterium* must be chosen for the emended genus (Principle 6 and Rule 44 of the Bacteriological Code), and the genus *Aureobacterium* is synonymous with the genus *Microbacterium* (Rule 24a of Bacteriological Code).

Emended description of the genus *Microbacterium* Orla-Jensen 1919 emend. Collins, Jones and Kroppenstedt 1983

In young cultures, cells are small irregular rods of about 0·5 μm in width by 0·7–2·0 μm in length. In old cultures, rods become shorter or spherical elements, but a marked rod–coccus cycle is not observed. Motile by lateral flagella or non-motile. Gram-positive. Colonies on suitable semi-solid media are yellowish white, yellow or orange. Obligately arobic. Slow and weak oxidative production of acid from some carbohydrates. Catalase-positive. Do not form endospores. Not acid-fast. Menaquinones are predominantly MK-11, MK-12 and MK-13, while MK-10 and MK-14 are minor components.

The major fatty acids are anteiso- and iso-methyl branched-chain acids: anteiso-$C_{15:0}$, anteiso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and unidentified glycolipids. Peptidoglycan: the peptide subunit consists of alanine, D-glutamic acid (plus hydroxyglutamic acid), and either L-lysine, L-ornithine or L-homoserine. The interpeptide bridge contains L-lysine or D-ornithine. Cell walls do not contain *meso*- or LL-diaminopimelic acid. Muramic acid occurs in the *N*-glycolyl form. No arabinogalactan. The G+C content of the DNA ranges from 66 to 72 mol%. The pattern of 16S rRNA signatures consists of nucleotides at positions 69–99 (G/A-T), 129 (C/T), 232 (G), 279 (T), 443–491 (C/T-G), 770–809 (G-C/G), 780 (A), 830–856 (T-A) and 929–1386 (A-T). Habitat: widely distributed in soil and clinical specimens. Type species: *Microbacterium lacticum* Orla-Jensen 1919. Type strain: IFO 14135^T (= ATCC 8180^T).

Table 4. Distinguishing characteristics of the genera of the family *Microbacteriaceae*

Data from previous studies (see references 5, 8, 11, 12, 35, 38, 41, 42, 43 and 44).

Genus	Motility	Catalase	Peptidoglycan			Fatty acid type‡	Major menaquinones§	G+C content (mol%)
			Group*	Diamino acid†	Acyl type			
Microbacterium as redefined	D	+	B	L-Lys/D-Orn	Glycolyl	S, A, I	MK-11, 12, 13, 14	65–72
Microbacterium	D	+	B	L-Lys	Glycolyl	S, A, I	MK-11, 12	66–69
Aureobacterium	D	+	B	D-Orn	Glycolyl	S, A, I	MK-11, 12, 13, 14	65–76
Agrococcus	–	+	B	DAB	Acetyl	S, A, I	MK-11, 12	74
Agromyces	–	–	B	DAB	Acetyl	S, A, I	MK-11, 12	71–76
Clavibacter	–	+	B	DAB	Acetyl	S, A, I	MK-9, 10	67–78
Cryobacterium	–	+	B	DAB	ND	S, A, I, 12-H	MK-10	65
Curtobacterium	D	+	B	D-Orn	Acetyl	S, A, I, (H)	MK-9	68–75
Leucobacter	–	+	B	DAB	Acetyl	S, A, I	MK-11	66·2
Rathayibacter	–	+	B	DAB	ND	S, A, I	MK-10	63–72

+, 90% or more of strains are positive; –, 90% or more of strains are negative; D, substantial proportion of species differ; ND, not determined.

* Designation of Schleifer & Kandler (30).

† DAB, diaminobutyric acid; Lys, lysine; Orn, ornithine.

‡ S, straight-chain saturated; A, anteiso-methyl branched; I, iso-methyl branched; (H), cyclohexyl fatty acids sometimes present; 12-H, 12-methyl-hexadecanoic acid.

§ Designations of Collins & Jones (3).

Transfer of *Aureobacterium* species to the genus *Microbacterium*

We transfer the *Aureobacterium* species to the genus *Microbacterium*: *Aureobacterium arabinogalactanolyticum* Yokota *et al.* 1993 (44) to *Microbacterium arabinogalactanolyticum* comb. nov., *Aureobacterium barkeri* Collins *et al.* 1983 (5) to *Microbacterium barkeri* comb. nov., *Aureobacterium esteraromaticum* (Omelianski 1923) (23) Yokota *et al.* 1993 (44) to *Microbacterium esteraromaticum* comb. nov., *Aureobacterium flavescens* (Lochhead 1958) (21) Collins *et al.* 1983 (5) to *Microbacterium flavescens* comb. nov., *Aureobacterium keratanolyticum* Yokota *et al.* 1993 (44) to *Microbacterium keratanolyticum* comb. nov., *Aureobacterium liquefaciens* Collins *et al.* 1983 (5) to *Microbacterium liquefaciens* comb. nov., *Aureobacterium luteolum* Yokota *et al.* 1993 (44) to *Microbacterium luteolum* comb. nov., *Aureobacterium saperdae* Yokota *et al.* 1993 (44) to *Microbacterium saperdae* comb. nov., *Aureobacterium schleiferi* Yokota *et al.* 1993 (44) to *Microbacterium schleiferi* comb. nov., *Aureobacterium terrae* Yokota *et al.* 1993 (44) to *Microbacterium terrae* comb. nov., *Aureobacterium terregens* (Lochhead and Burton 1953) (22) Collins *et al.* 1983 (5) to *Microbacterium terregens* comb. nov., *Aureobacterium testaceum* (Komagata and Iizuka 1964) (19) Collins *et al.* 1983 (5) to *Microbacterium testaceum* comb. nov., and *Aureobacterium trichothecenolyticum* Yokota *et al.* 1983 (44) to *Microbacterium trichothecenolyticum* comb. nov.

The validated species *Flavobacterium marinotypicum* strain IFO 15779, the non-valid species '*Aureobacterium ketoreductum*' strains IFO 14548 and IFO 14549, '*Chromobacterium chocolatum*' strain IFO 3758, two strains of CDC group A-4 and A-5 (strains IFO 15708 and IFO 15709), and three isolates from mangrove rhizosphere (strains IFO 16060, IFO 16061 and IFO 16062) are thought to be new species in the redefined genus *Microbacterium* (37) based on their physiological, chemotaxonomic and phylogenetic features.

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