NOTE

***Microbacterium aerolatum* sp. nov., isolated from the air in the 'Virgilkapelle' in Vienna**

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Three rod-shaped, Gram-positive strains were isolated from the air of the chapel 'Virgilkapelle' in Vienna. A representative of these three strains, strain V-73^T, shared the highest 16S rDNA sequence similarities with members of the genus *Microbacterium*, in particular *Microbacterium foliorum*, *Microbacterium testaceum*, *Microbacterium esteraromaticum*, *Microbacterium keratanolyticum* and *Microbacterium arabinogalactanolyticum*. The strains displayed almost identical biochemical and physiological characteristics and showed no differences in their protein patterns obtained after SDS-PAGE. On the basis of Fourier-transform infra-red (FT-IR) spectra and genomic fingerprints, the three strains were grouped together and separated from the other relevant members of the genus *Microbacterium*. The chemotaxonomic characteristics analysed, including polar lipids, quinone systems, cell wall composition and fatty acid profiles, were in good agreement with the characteristics described for the genus *Microbacterium*. The G+C content of the DNAs was determined to be in the narrow range 69.3–69.7 mol %. The results of DNA–DNA hybridization, biochemical/physiological characterization, ERIC-PCR-generated genomic fingerprints and FT-IR spectra demonstrated that the three isolates represent a novel species of the genus *Microbacterium*. The name *Microbacterium aerolatum* sp. nov. is proposed for the novel species, of which strain V-73^T (= DSM 14217^T = CCM 4955^T) is the type strain.

Keywords: *Microbacterium aerolatum* sp. nov., 16S rDNA sequence, chemotaxonomy, biochemical/physiological traits

The genus *Microbacterium* has been emended recently to combine the genera *Microbacterium* and *Aureobacterium* (Takeuchi & Hatano, 1998a). In spite of high 16S rDNA sequence similarities, members of the genus are heterogeneous in terms of chemotaxonomic characteristics such as quinone systems, peptidoglycan types and polyamine patterns (Yokota *et al.*, 1993a, b; Takeuchi & Hatano, 1998a, b; Altenburger *et al.*, 1997; Matsuyama *et al.*, 1999). Species described so far have been isolated from the phyllosphere of grasses, soil, sewage, lake water, steep liquor, milk products or cheese and clinical specimens (Breid, 1957; Collins *et al.*, 1983; Collins & Keddle, 1986; Yokota *et al.*, 1993a, b; Saweljew *et al.*, 1996; Takeuchi & Hatano, 1998a, b; Behrendt *et al.*, 2001). In a study concerning the bacteriological biodiversity in the air of the chapel

'Virgilkapelle' underneath the 'Stephansplatz' in Vienna, among others, three yellow-pigmented, coryneform bacteria were isolated. Here, we describe the classification of these strains, which could be clearly allocated to the genus *Microbacterium*. The three strains, designated V-73^T, V-62 and V-72, were characterized using a set of methods summarized by Zlamala *et al.* (2002) and Wieser & Busse (2000).

Colonies of the three isolates V-73^T, V-62 and V-72 were translucent, yellow-pigmented, opaque, circular, low-convex, moist and 2–7 mm in diameter. Colony margins were entire on nutrient agar. Cells were small, slender rods and occurred singly or in irregular clusters. Mycelial growth and a marked rod–coccus cycle were not observed. The three isolates showed Gram-positive behaviour in Gram-staining and KOH test. They grew best on the complex organic medium tryptic soy agar (TSA) at temperatures ranging from 18 to 28 °C and in the presence of 4% NaCl. Good

The EMBL accession number of the 16S rDNA sequence of *Microbacterium aerolatum* V-73^T is AJ309929.

Table 1. Physiological and biochemical properties of *M. aerolatum*, *M. esteraromaticum* and *M. arabinogalactanolyticum*

Properties were analysed as described by Kämpfer *et al.* (1991) and Zlamala *et al.* (2002). +, Positive; —, negative; d, some strains positive; w, weak reaction. Symbols (†) indicate results that are not in line with those reported previously by Yokota *et al.* (1993b). All five strains were negative for decomposition of Tween 80† and urea, † acid production from D-ribose, D-melibiose, D-inulin, D-raffinose, inositol, dulcitol and sorbitol, utilization of α -D-melibiose, adonitol, *i*-inositol, D-sorbitol, *trans*-aconitate, adipate, azelate, citrate, † itaconate, mesaconate, suberate, β -alanine and L-tryptophan and hydrolysis of aesculin, *p*-nitrophenyl (pNP) β -D-glucuronide, 2-deoxythymidine-5'-pNP phosphate and L-glutamate- γ -3-carboxy *p*-nitroanilide (pNA). All five strains were negative in the Voges-Proskauer, methyl red, † oxidase and indole tests as well as in the production of H₂S†. All five strains were positive for decomposition of potato starch and utilization of L-arabinose, *N*-acetyl D-glucosamine, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, sucrose, D-trehalose, acetate, propionate, DL-3-hydroxybutyrate, pyruvate, L-histidine, L-ornithine and L-proline and hydrolysis of pNP α -D-glucopyranoside, pNP β -D-glucopyranoside, bis-pNP phosphate, pNP phenylphosphonate, L-alanine pNA and L-proline pNA. All five strains grow in the presence of 2%† and 4% NaCl, but not 10% NaCl. All five strains grow well at 28 °C, but not at 42 °C.

Characteristic	<i>M. aerolatum</i> (3 strains)	<i>M. esteraromaticum</i> DSM 8609 ^T	<i>M. arabinogalactanolyticum</i> DSM 8611 ^T
Catalase	+	—	+
Nitrate reduction	+	—	+
Acid production from:			
D-Glucose	+	—	+
D-Trehalose	—	—	+
L-Arabinose	+	—	w
D-Sucrose	+	—	+
D-Galactose	+	—	w
D-Mannose	+	—	+
D-Maltose	+	—	+
L-Rhamnose	+	—	+
D-Lactose	—	—	w
D-Mannitol	+	—	+
D-Xylose	+	—	—
Glycerol	—	—	+
Utilization of:			
<p>-Arbutin</p>	—	+	+
L-Rhamnose	+	—	+
D-Ribose	+	—	+
Salicin	—	+	+
Maltitol	+	+	—
D-Xylose	+	—	+
D-Mannitol	—	+	+
Putrescine	—	+	+
<i>cis</i> -Aconitate	d (1/3)*	—	—
4-Aminobutyrate	+	+	—
Fumarate	—	+	+
Glutarate	—	+	—
DL-Lactate	+	+	—†
L-Malate	—	—	+
Oxoglutarate	+	—	—
L-Alanine	—	+	+
L-Aspartate	+	—	—
L-Leucine	+	—	+
L-Phenylalanine	—	—	+
L-Serine	—	—	+
3-Hydroxybenzoate	+	—	—
4-Hydroxybenzoate	+	—	—
Phenylacetate	—	—	+
Tyrosine decomposition	—	+	—
Hydrolysis of:			

Table 1 (cont.)

Characteristic	<i>M. aerolatum</i> (3 strains)	<i>M. esteraromaticum</i> DSM 8609 [†]	<i>M. arabinogalactanolyticum</i> DSM 8611 [†]
pNP β -D-galactopyranoside	—	—	+
pNP phosphorylcholine	—	—	+
Gelatin	—	—	+
Casein	—	—	+
Tween 20	—	+	—
Growth at/in:			
6.5% NaCl	+	w	w
4 °C	+	w	—
37 °C	+	+	—

* Strain V-62 positive.

† Data not in line with Yokota *et al.* (1993b).

growth was observed at 37 °C and in the presence of 6.5% NaCl. There was weak growth at 4 °C and in the presence of 10% NaCl. No growth occurred at 42 °C or under microaerobic conditions. Physiological and biochemical characteristics of the three isolates are summarized in Table 1. Of more than 100 physiological and biochemical characteristics, the three strains differed only in the utilization of *cis*-aconitate. Almost identical protein patterns (results not shown) supported the assumption that the three strains V-73^T, V-62 and V-72 are members of a single species. The G+C contents of the genomic DNA of the three isolates, as determined by the method described by Cerdà-Cuellar *et al.* (1997), were in the narrow range 69.3–69.7 mol%.

Analysis of the 16S rDNA of strain V-73^T, the representative of this group, resulted in a fragment of 1402 bases (positions 50–1469, *Escherichia coli* numbering; Brosius *et al.*, 1978). Sequence comparisons showed that strain V-73^T shared the highest similarities with *Microbacterium foliorum* DSM 12966^T (98.1%), *Microbacterium testaceum* DSM 20166^T (98.0%), *Microbacterium esteraromaticum* DSM 8609^T (97.7%), *Microbacterium arabinogalactanolyticum* DSM 8611^T (97.6%) and *Microbacterium keratanolyticum* DSM 8606^T (97.6%). Sequence similarities to other species of the genus *Microbacterium* were in the range 97.5–95.0% and to other species of the family *Microbacteriaceae*, were in the range 94.0–91.0%. This result demonstrated clearly that V-73^T is affiliated to the genus *Microbacterium* (Takeuchi & Hatano, 1998a). Signature nucleotides detected in the 16S rDNA sequence of strain V-73^T were in agreement with those used for the description of the genus (Takeuchi & Hatano, 1998a). The close relationship of V-73^T to *M. esteraromaticum* was supported by the detection of the signature adenosine at position 69 of the 16S rDNA sequence (*E. coli* numbering; Brosius *et al.*, 1978), so far described only for *M. esteraromaticum* DSM 8609^T (accession no.

Y17231; Schumann *et al.*, 1999) and *Microbacterium barkeri* DSM 20145^T (accession no. X77446; Rainey *et al.*, 1994) (Takeuchi & Hatano, 1998a). This signature was also found in the 16S rDNA sequence of *M. arabinogalactanolyticum* DSM 8611^T (accession no. Y17228; Schumann *et al.*, 1999).

Chemotaxonomic characteristics confirmed the placement of the three isolates in a single species and also their identification as members of the genus *Microbacterium*. The peptidoglycan of strain V-73^T contained the following amino acids: ornithine, alanine, glycine, glutamic acid, hydroxyglutamic acid (Hyr) and homoserine (Hsr). From these data and from the occurrence of the peptides Gly → D-Glu, Gly → D-Orn, D-Ala → D-Orn and D-Ala → D-Orn ← Gly in the partial hydrolysate of the peptidoglycan (data not shown), we concluded that the peptidoglycan type is B2 β (Schleifer & Kandler, 1972) D-Glu(Hyr) → Gly → D-Orn, with Gly at position 1 and L-Hsr at position 3 of the peptide subunit (type B6; DSMZ, 2001). The quinone systems of strains V-73^T, V-62 and V-72, which were analysed according to Tindall (1990), consisted of the major menaquinones MK-12 (56–64%) and MK-13 (30–44%). Minor amounts of menaquinone MK-11 (1–6%) were also detected. Fatty acid composition was analysed as described by Kämpfer *et al.* (1997). The predominant fatty acids in V-73^T were ai-C_{15:0} and ai-C_{17:0}, with significant amounts of i-C_{16:0} (Table 2). Whereas the fatty acid profiles of V-73^T and *M. testaceum* DSM 20166^T were similar in their major characteristics, quantitative differences in the contents of i-C_{15:0} and ai-C_{17:0} distinguished V-73^T from *M. esteraromaticum* DSM 8609^T, *M. arabinogalactanolyticum* DSM 8611^T and *M. keratanolyticum* DSM 8606^T (Table 2) and from the profile reported for *M. foliorum* DSM 12966^T (Behrendt *et al.*, 2001).

Polar lipid profiles were analysed by TLC (Ventosa *et al.*, 1993). Diphosphatidylglycerol and phosphatidylglycerol were found to be predominant in polar lipid

Table 2. Fatty acid composition of *M. aerolatum* sp. nov. and related species

Strains: 1, *M. aerolatum* V-73^T; 2, *M. testaceum* DSM 20166^T; 3, *M. esteraromaticum* DSM 8609^T; 4, *M. keratanolyticum* DSM 8606^T; 5, *M. arabinogalactanolyticum* DSM 8611^T. Values are percentages of total fatty acids. tr, Trace.

Fatty acid	1	2	3	4	5
ai-C _{13:0}				tr	0.3
i-C _{14:0}	0.5	0.9	3.2	7.5	4.8
C _{14:0}		0.7	0.4		0.8
i-C _{15:1}				0.2	
ai-C _{15:1}		1.4	1.2	0.7	0.4
i-C _{15:0}	1.8	1.9	11.9	13.5	15.2
ai-C _{15:0}	44.1	40.1	68.5	44.8	49.6
C _{15:0}		0.8		0.5	
C _{16:1} iso G		0.6			
i-C _{16:0}	13.0	18.5	12.3	27.4	17.4
C _{16:0}	3.8		2.5	0.6	2.0
ai-C _{17:1}		1.7			
i-C _{17:0}	0.7	0.8		1.6	1.3
ai-C _{17:0}	36.0	22.8	8.1	2.7	7.9

extracts of strains V-73^T, V-62 and V-72. In addition, two unknown glycolipids were detected. These combined chemotaxonomic characteristics are in agreement with those reported for *M. esteraromaticum* and *M. arabinogalactanolyticum* (Yokota *et al.*, 1993b), two of the most closely related species as indicated from 16S rDNA sequence comparisons. In contrast, some of these characteristics distinguished the isolates from *M. keratanolyticum* DSM 8606^T, which was reported to have a quinone system with nearly equal amounts of MK-12 and MK-13 but a cell wall of the peptidoglycan type B2 α , as well as from *M. testaceum* DSM 20166^T, which contains MK-11 as the major menaquinone (Yokota *et al.*, 1993b), and from *M. foliorum* DSM 12966^T, with MK-11 and MK-12 as major menaquinones (Behrendt *et al.*, 2001). Therefore, the 16S rDNA sequence comparison in combination with the quinone system and peptidoglycan type indicated a possible relationship only with *M. esteraromaticum* and *M. arabinogalactanolyticum*. In conclusion, the three strains were compared only to *M. esteraromaticum* DSM 8609^T and *M. arabinogalactanolyticum* DSM 8611^T to determine their taxonomic status.

DNA relatedness among V-73^T, V-62 and V-72 and with *M. esteraromaticum* DSM 8609^T and *M. arabinogalactanolyticum* DSM 8611^T was analysed according to Lind & Ursing (1986) and Ziemke *et al.* (1998). The three strains shared 100% DNA relatedness among themselves, whereas only low reassociation values (28.9–48.8%) were obtained between the isolates and the reference strains *M. esteraromaticum* DSM 8609^T and *M. arabinogalactanolyticum* DSM 8611^T. These

Fig. 1. Negative image of ERIC-PCR-generated genomic fingerprints of strains V-62 (lane 2), V-72 (3), V-73^T (4), *M. esteraromaticum* DSM 8609^T (5) and *M. arabinogalactanolyticum* DSM 8611^T (6). Lanes 1 and 7, size markers (λ DNA BstEII digest); lane 8, negative control.

Fig. 2. Average linkage dendrogram based on the distances of first derivatives of FT-IR spectra (frequency ranges 3000–2800 cm⁻¹, 1201–900 cm⁻¹, 901–699 cm⁻¹).

data demonstrate unambiguously that V-73^T, V-62 and V-72 are members of a single novel species of the genus *Microbacterium*. The three isolates could be also distinguished from *M. esteraromaticum* DSM 8609^T and *M. arabinogalactanolyticum* DSM 8611^T on the basis of their characteristic genomic fingerprints obtained after ERIC-PCR (Wieser & Busse, 2000) and FT-IR spectra (Wieser *et al.*, 2002). A high degree of similarity in genomic fingerprints (Fig. 1) was detected for the three strains. However, strains V-62 and V-72 displayed identical fingerprints, indicating a clonal relationship, whereas strain V-73^T could be distinguished from strains V-62 and V-72 on the basis of several different bands. Analysis of FT-IR spectra showed that the three isolates V-73^T, V-62 and V-72 were grouped in a cluster and separate from the two closely related species *M. esteraromaticum* and *M. arabinogalactanolyticum* (Fig. 2).

From the results presented here, it becomes evident that the three strains V-73^T, V-62 and V-72 represent a single novel species of the genus *Microbacterium* that can be distinguished from chemotaxonomically similar species of the genus such as *M. esteraromaticum* and *M. arabinogalactanolyticum* on the basis of genomic fingerprints (Fig. 1), FT-IR spectra (Fig. 2) and more than 25 biochemical/physiological characteristics (Table 1). Thus, we propose the name *Microbacterium aerolatum* sp. nov. for the novel species.

Description of *Microbacterium aerolatum* sp. nov.

Microbacterium aerolatum (ae.ro.la'tum. Gr. n. *aer* air, L. part. adj. *latum* carried, N.L. part. adj. *aerolatum* airborne).

Cells are small rods. The cells vary in size from 0.5 to 0.7 µm wide by 1.5 to 1.9 µm long. Cells occur singly or in irregular clusters. They are Gram-positive and non-motile. Endospores are not observed. Colonies are circular, slightly convex, opaque and moist. Colony diameters on TSA are 2–7 mm. The colour of the colonies on nutrient agar is yellow. Aerobic. Catalase is produced. Oxidase-negative. Nitrate is reduced to nitrite. Physiological and biochemical traits are shown in Table 1. The cell wall diamino acid is D-ornithine and the peptidoglycan type is B2β containing the amino acids ornithine, alanine, glycine, glutamic acid, hydroxyglutamic acid and homoserine. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol and two unknown glycolipids. The fatty acid profile consists of the predominant compounds ai-C_{15:0}, ai-C_{17:0} and i-C_{16:0}, and i-C_{15:0} is present in minor amounts. The quinone system consists of the major menaquinones MK-12 and MK-13. The G + C content is 69.3–69.7 mol%.

The type strain, V-73^T (= DSM 14217^T = CCM 4955^T), was isolated from the air in the chapel 'Virgilkapelle' in Vienna, Austria.

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