Promicromonospora vindobonensis sp. nov. and *Promicromonospora aerolata* sp. nov., isolated from the air in the medieval 'Virgilkapelle' in Vienna

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Two airborne bacterial isolates designated V45^T and V54A^T were characterized in order to determine their taxonomic position. 16S rDNA sequence analysis showed that the two isolates shared 98.1 % sequence similarity. Highest sequence similarities (98.0–98.5 %) were found to *Promicromonospora citrea* DSM 43110^T and *Promicromonospora sukumoe* IFO 14650^T. Detection of a quinone system with the predominant compound MK-9(H₄), a polar lipid pattern containing phosphatidylglycerol, a fatty acid profile with the predominant acids C_{15:0} iso and C_{15:0} anteiso and the diagnostic cell-wall diamino acid L-lysine supported the assignment of the novel isolates to the genus *Promicromonospora*. The two isolates could be distinguished from *P. sukumoe* by the presence of glycine in the peptidoglycan, and the detection of the cell-wall sugar galactose differentiates them from the two established species of the genus *Promicromonospora*. Each of the two isolates displayed a unique biochemical profile. Results from DNA–DNA hybridizations clearly demonstrated that V45^T and V54A^T represent separate species. Based on these data, it is proposed that V45^T (= IFO 16525^T = CCM 7044^T) and V54A^T (= IFO 16526^T = CCM 7043^T) be classified as the type strains of two novel *Promicromonospora* species, for which the names *Promicromonospora vindobonensis* sp. nov. and *Promicromonospora aerolata* sp. nov. are proposed.

In a study concerning bacterial diversity in the air of the medieval 'Virgilkapelle' chapel beneath the 'Stephansplatz' square in Vienna, airborne bacteria were collected using a BIOTEST Hycon RCS Plus air-sampler and subcultivated on PYES agar (Zlamala *et al.*, 2002a). Strains displaying similar colony types were compared to each other by their protein patterns obtained after SDS-PAGE (Altenburger *et al.*, 1996). If protein patterns indicated a relationship at the species level, representatives were selected for further

characterization employing partial 16S rDNA sequencing and analysis of chemotaxonomic characteristics including polyamine patterns, polar lipid profiles, cell-wall diamino acids, fatty acids and quinone systems. Based on their classification, the isolates were identified as members of the genera *Arthrobacter*, *Promicromonospora*, *Moraxella* (C. Zlamala and H.-J. Busse, unpublished results), *Agrococcus* (Zlamala *et al.*, 2002a) and *Microbacterium* (Zlamala *et al.*, 2002b). Here, we report the detailed characterization of two *Promicromonospora* isolates designated V45^T and V54A^T that did not display any similarities in their protein patterns.

The genus *Promicromonospora* currently consists of two species, *Promicromonospora citrea* (Krasilnikov *et al.*, 1961) and *Promicromonospora sukumoe* (Takahashi *et al.*, 1987). A third species, *Promicromonospora enterophila*, has been transferred to the re-established genus *Oerskovia* as *Oerskovia enterophila* (Stackebrandt *et al.*, 2002). Although reported to

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The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains V45^T and V54A^T are AJ487302 and AJ487303.

DNA–DNA hybridization results and micrographs of cells of the novel strains are available as supplementary material in IJSEM Online.

form monospores (Krasilnikov *et al.*, 1961), this observation was not confirmed by other studies (Lechevalier & Lechevalier, 1981). Species of the genus are characterized by the production of mycelia that fragment into bacillary or coccoid elements. Phylogenetically, the genus *Promicromonospora* is most closely related to the genera *Rarobacter*, *Cellulosimicrobium*, *Oerskovia* and *Cellulomonas* (Schumann *et al.*, 2001).

16S rDNA studies

16S rRNA-encoding genes were analysed as described by Zlamala *et al.* (2002a). The resulting 16S rDNA sequences of V45^T and V54A^T respectively consisted of 1342 (positions 81–1444; *Escherichia coli* numbering) and 1393 (29–1442; *E. coli* numbering) nucleotides. In a FASTA search (Pearson & Lipman, 1988), the two isolates shared 98.1% sequence similarity. Isolates V45^T and V54A^T exhibited highest sequence similarities (98.3–98.4% and 98.1–98.3%, respectively) to the type strains of the two established species *P. citrea* (DSM 43110^T) and *P. sukumoe* (IFO 14650^T). The 16S rDNA sequences were aligned with published sequences available from DDBJ, EMBL and GenBank. Phylogenetically, the two isolates were located on the *Promicromonospora* lineage and the degree of branching was supported by high bootstrap values (Fig. 1).

DNA studies

The G + C content of strains V45^T and V54A^T was analysed according to the methods of Mesbah *et al.* (1989) and Tamaoka & Komagata (1984) and determined to be 70 mol%. DNA–DNA hybridization experiments were carried out as described previously (Ezaki *et al.*, 1989). The results (Supplementary Table A, available in IJSEM

Online) demonstrated that strains V45^T and V54A^T represent two separate, previously unrecognized species.

Chemotaxonomic characteristics

The quinone systems of both isolates, analysed according to the procedure of Ventosa *et al.* (1993), contained the predominant menaquinone MK-9(H₄). In addition, the quinone system of strain V45^T contained 4% MK-9(H₂) and 4% MK-9(H₆) and the quinone system of V54A^T contained 7% MK-9(H₂). Polar lipids were analysed as described by Tindall (1990). The polar lipid profile of V45^T consisted of diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, three unknown phosphoglycolipids, one unknown phospholipid and one unknown polar lipid. The polar lipid profile of V54A^T consisted of diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, two unknown phosphoglycolipids and one unknown phospholipid. Both the quinone systems and the polar lipid compositions are in excellent agreement with the characteristics reported for representatives of the genus *Promicromonospora* (Kalakoutskii *et al.*, 1989; Takahashi *et al.*, 1987). Fatty acids were analysed as described by Kämpfer & Kroppenstedt (1996). Both isolates had a fatty acid profile similar to that of *P. citrea* IFO 12397^T and *P. sukumoe* IFO 14650^T, which contained the predominant acids C_{15:0} anteiso and C_{15:0} iso and moderate amounts of C_{16:0} and C_{17:0} anteiso (Table 1). The two isolates were only distinguishable from each other and from reference strains of the genus by quantitative differences and the presence/absence of some minor acids. Cell walls were prepared and analysed according to Schleifer & Kandler (1972) and amino acid and sugar compositions were identified by HPLC as described by Takeuchi *et al.* (1999). The cell wall of V45^T consisted of Glu:Gly:Ala:Lys (0.98:0.69:2.88:1.0) and that of V54A^T consisted of Glu:Gly:Ala:Lys (1.05:0.43:3.4:1.0). Asp and Ser could not be detected in their cell walls. Based on these results, the structure of the linkage may be Gly–Ala, suggesting peptidoglycan type A3α (Schleifer & Kandler, 1972). The cell-wall sugars of strains V45^T and V54A^T were rhamnose (respectively 14 and 4%), galactose (both 37%) and glucose (49 and 59%). The presence of glycine in the peptidoglycan distinguishes the two isolates from *P. sukumoe* IFO 14650^T, and the presence of the cell-wall sugar galactose has not been reported for either species of *Promicromonospora* (Takahashi *et al.*, 1987).

Analysis of the polyamine patterns of strains V45^T, V54A^T, *P. citrea* IFO 12397^T and *P. sukumoe* IFO 14650^T was done as described by Altenburger *et al.* (1997). Three strains (V45^T, V54A^T and *P. citrea* IFO 12397^T) contained similar polyamine patterns, consisting of the major compound spermidine [5.6, 3.5 and 2.7 μmol (g dry weight)⁻¹, respectively] and moderate to minor amounts of spermine [1.7, 1.3 and 0.5 μmol (g dry weight)⁻¹] and putrescine [0.3, 0.1 and 0.5 μmol (g dry weight)⁻¹]. Traces of 1,3-diaminopropane and cadaverine were also detected. In contrast, *P. sukumoe* IFO 14650^T exhibited a polyamine

Fig. 1. Dendrogram based on 16S rDNA sequences showing the phylogenetic relationships of *Promicromonospora* species and related genera. The dendrogram was constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) and clustering with maximum-parsimony method were performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are given at branching points.

Table 1. Major fatty acid components of *Promicromonospora* strains

Strains: 1, *P. vindobonensis* sp. nov. V45^T; 2, *P. aerolata* sp. nov. V54A^T; 3, *P. sukumoe* IFO 14650^T; 4, *P. citrea* IFO 12397^T. Values are percentages of total fatty acids. Isolates V45^T and V54A^T were grown at 28 °C on GYM agar (l⁻¹ distilled water: 4 g glucose, 4 g yeast extract, 10 g malt extract, 2 g CaCO₃, 12 g agar); the remaining strains were grown on trypticase soy agar at 28 °C prior to analysis. Fatty acid nomenclature: the position of the double bond in an unsaturated fatty acid can be located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*. Summed feature 5 contains 18:2 ω 6,9*c* and/or 18:0 anteiso, which could not be separated by GLC with the MIDI system.

Compound	1	2	3	4
C _{14:0}	1.5	0.8	1.3	0.6
C _{14:0} iso	—	0.3	0.9	1.2
C _{15:1} iso G	—	0.4	3.4	—
C _{15:1} anteiso A	—	0.4	4.4	—
C _{15:0} iso	36.7	30.8	36.6	40.6
C _{15:0} anteiso	46.5	51.1	38.0	38.9
C _{15:0}	—	—	1.6	1.0
C _{16:0} N alcohol	—	—	0.4	—
C _{16:0} iso	2.2	2.2	3.9	4.0
C _{16:0}	6.0	3.8	2.4	0.8
Summed feature 5	2.0	1.4	—	—
C _{17:0} iso	1.3	1.5	2.0	3.1
C _{17:0} anteiso	3.8	6.7	4.6	9.7
C _{18:1} ω 9 <i>c</i>	—	0.5	—	—
C _{18:3} ω 6 <i>c</i> (6,9,12)	—	—	0.4	—

pattern in which the concentrations of the major polyamines were two orders of magnitude lower, clearly distinguishing it from the other three strains. These results indicate that at least two different polyamine patterns can be found in the genus *Promicromonospora*. Similar polyamine patterns have already been reported for representatives of related genera such as *Cellulomonas*, *Cellulosimicrobium* and *Oerskovia* (Hamana, 1995).

Biochemical characteristics

Investigation of *P. citrea* IFO 12397^T, *P. sukumoe* IFO 14650^T, V45^T and V54A^T revealed metabolic profiles that were distinct for each strain (Table 2). They could be distinguished from each other easily by their biochemical profiles, supporting the idea that each of strains V45^T and V54A^T represents a distinct species. Gram-staining (Moaledji, 1986) of cells of the two isolates revealed Gram-positive behaviour.

Taxonomic considerations

16S rDNA sequence comparisons and chemotaxonomic characteristics of the isolates suggest assignment of strains V45^T and V54A^T to the genus *Promicromonospora*. Results

from DNA–DNA hybridizations, as well as unique physiological profiles, demonstrate that each of strains V45^T and V54A^T represents a separate species within the genus.

Since the four species of the genus *Promicromonospora* exhibit not more than 98.5 % 16S rDNA sequence similarity to each other, in future studies, highest values below 98.6 % can be considered to suggest the detection of a novel species of the genus *Promicromonospora*.

Description of *Promicromonospora vindobonensis* sp. nov.

Promicromonospora vindobonensis (vin.do.bo.nen'sis. L. fem. adj. *vindobonensis* of *Vindobona*, the Roman name for Vienna, where the type strain was isolated.)

Cells show branching hyphae (Supplementary Fig. A, available in IJSEM Online) that are 0.3–0.5 μ m in diameter after 18 h of growth on PY agar. Cells stain Gram-positive. After 3 days of growth, the mycelium fragments into non-motile, Y- or V-shaped, rod-like, coccoid elements, 0.3–0.5 $\times$ 0.6–1.5 μ m. No sessile, oval spores, chlamydospore-like or other spore-like elements are observed. On PY agar, colonies are whitish, translucent, convex, glistening and approx. 1 mm in diameter with an entire edge. Physiological and biochemical characteristics are summarized in Table 2. In the peptidoglycan, glutamic acid, glycine, alanine and lysine are present. Cell-wall sugars are glucose, galactose and rhamnose. Diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, three unknown phosphoglycolipids, one unknown phospholipid and one unknown polar lipid are present in the polar lipid profile. Predominant acids in the fatty acid profile are C_{15:0} anteiso and C_{15:0} iso. C_{16:0} and C_{17:0} anteiso are present in moderate amounts. The quinone system consists of the major compound MK-9(H₄). Spermidine is the predominant cellular polyamine and spermine is present in moderate amounts. The G+C content is 70 mol%.

The type strain, V45^T (= IFO 16525^T = CCM 7044^T), was isolated from the air in the 'Virgilkapelle' chapel in Vienna.

Description of *Promicromonospora aerolata* sp. nov.

Promicromonospora aerolata (a.e.ro.la'ta. Gr. n. *aer* air; L. part. adj. *latum*, -a carried; N.L. part. fem. adj. *aerolata* airborne).

Cells show branching hyphae (Supplementary Fig. B, available in IJSEM Online) that are 0.3–0.5 μ m in diameter after 18 h of growth on PY agar. Cells stain Gram-positive. After 3 days of growth, the mycelium fragments into non-motile, Y- or V-shaped, rod-like, coccoid elements, 0.3–0.5 $\times$ 0.6–1.5 μ m. No sessile, oval spores, chlamydospore-like or other spore-like elements are observed. Colonies are light yellow, translucent and reach approx. 1 mm in diameter. Physiological and biochemical characteristics are summarized in Table 2.

Table 2. Biochemical characteristics of *Promicromonospora* strains

Strains: 1, *P. vindobonensis* sp. nov. V45^T; 2, *P. aerolata* sp. nov. V54A^T; 3, *P. sukumoe* IFO 14650^T; 4, *P. citrea* IFO 12397^T. +, Positive; (+), weakly positive; −, negative; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl. All strains are positive for aesculin (β-glucosidase), gelatinase, catalase and pyrazinamidase (API Coryne). All strains are negative for alkaline phosphatase, β-glucuronidase, α-glucosidase, β-galactosidase and *N*-acetyl-β-glucosaminidase (API Coryne), fermentation of ribose, xylose, mannitol and lactose and production of acid from erythritol, D-arabinose, L-arabinose, ribose, xylose, adonitol, methyl β-xyloside, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α-mannoside, lactose, melibiose, inulin, raffinose, starch, xylitol, gentiobiose, lyxose, tagatose, fucose, arabitol, gluconate, 2-ketogluconate and 5-ketogluconate (API 50CH).

Characteristic	1	2	3	4
API Coryne tests:				
Nitrate reductase	+	−	+	−
Pyrrolidonyl arylamidase	+	+	(+)	(+)
Urease	−	−	+	(+)
Fermentation of glucose, maltose, sucrose, glycogen	−	+	−	−
Acid production from (API 50CH):				
Glycerol, methyl α-glucoside, salicin, sucrose, arbutin, maltose, turanose, amygdalin, melezitose, D-mannose, galactose, D-glucose, trehalose, glycogen	−	+	−	−
D-Fructose	+	+	−	−
Cellobiose	−	+	+	−
Mannitol, <i>N</i> -acetylglucosamine	−	(+)	−	−
Assimilation of:*				
<i>N</i> -Acetyl-D-glucosamine, D-trehalose, acetate	+	(+)	+	+
D-Cellobiose, D-fructose	(+)	+	+	+
D-Glucose, D-maltose, D-xylose	+	−	+	+
D-Mannose, D-ribose, sucrose, D-sorbitol, salicin	(+)	−	+	−
L-Arabinose, L-aspartate, L-histidine	−	−	+	+
Pyruvate	(+)	(+)	+	(+)
L-Proline	+	(+)	+	(+)
DL-3-Hydroxybutyrate, DL-lactate, L-malate	(+)	(+)	+	+
Fumarate	(+)	−	+	+
L-Alanine	−	−	+	(+)
L-Rhamnose	(+)	(+)	−	+
Glutarate	(+)	(+)	−	−
D-Melibiose, <i>cis</i> -aconitate, citrate	−	−	+	−
Propionate	+	−	−	−
Hydrolysis of:*				
pNP phenylphosphonate, L-proline pNA, L-alanine pNA, pNP β-D-xylopyranoside, pNP β-D-glucopyranoside	+	−	+	+
Aesculin	(+)	−	+	+
bis-pNP phosphate	−	−	+	+
pNP β-D-galactopyranoside	−	−	(+)	(+)

*Determined according to Kämpfer *et al.* (1991).

The peptidoglycan contains glutamic acid, glycine, alanine and lysine. Cell-wall sugars are glucose, galactose and rhamnose. Diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, two unknown phosphoglycolipids and one unknown phospholipid are present in the polar lipid profile. Predominant acids in the fatty acid profile are C_{15:0} anteiso and C_{15:0} iso. C_{16:0} and C_{17:0} anteiso are present in moderate amounts. The quinone system consists of the major compound MK-9(H₄). Spermidine is the predominant cellular polyamine and spermine is present in moderate amounts. The G + C content is 70 mol%.

The type strain, V54A^T (= IFO 16526^T = CCM 7043^T), was isolated from the air in the ‘Virgilkapelle’ chapel in Vienna.

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