Description of two novel species, *Sphingomonas abaci* sp. nov. and *Sphingomonas panni* sp. nov.

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Two Gram-negative, rod-shaped, non-spore-forming bacterial strains designated C42^T and C52^T were isolated in the Medical Clinic for Small Animals and Ungulates at the University for Veterinary Medicine Vienna, Austria. On the basis of 16S rRNA gene sequence similarity, both strains were shown to belong to the genus *Sphingomonas*. Strain C42^T showed the greatest levels of sequence similarity with *Sphingomonas melonis* DSM 14444^T and *Sphingomonas aquatilis* KCTC 2881^T (both 97.7 %). Strain C52^T showed the greatest levels of sequence similarity with *Sphingomonas koreensis* KCTC 2882^T (97.2 %), *Sphingomonas aquatilis* KCTC 2881^T (97.1 %) and *S. melonis* DSM 14444^T (97.0 %). The presence of Q-10 as the main ubiquinone, the predominance of the compound *sym*-homospermidine in the polyamine patterns, the presence of a *Sphingomonadaceae*-specific sphingoglycolipid in the polar lipid patterns, the presence of the fatty acid 2-OH C_{14:0} and the lack of 3-hydroxy fatty acids supported the identification of the two novel strains as members of the genus *Sphingomonas sensu stricto*. Unique physiological characteristics, protein patterns, quantitative differences in their fatty acid profiles and the results of genomic fingerprinting and DNA–DNA hybridizations differentiated strains C42^T and C52^T from closely related *Sphingomonas* species. Hence, the two strains are described as novel species of the genus *Sphingomonas sensu stricto*. The names *Sphingomonas abaci* sp. nov. (type strain C42^T=LMG 21978^T=DSM 15867^T) and *Sphingomonas panni* sp. nov. (type strain C52^T=LMG 21979^T=DSM 15761^T) are proposed.

During investigations designed to evaluate hygiene at the Medical Clinic for Small Animals and Ungulates at the University for Veterinary Medicine Vienna, Austria, numerous micro-organisms were isolated. In order to identify significant groups, isolates showing obvious similarities in colony morphology and pigmentation were subjected to comparison of their protein patterns after SDS-PAGE (Altenburger *et al.*, 1996). From protein-similarity groups consisting of at least three strains, a representative was characterized in more detail. On the basis of a preliminary classification, selected representatives of these protein-similarity groups were identified as members of the yeast genus *Rhodotorula*, the bacterial genera *Pseudomonas* (Hauser *et al.*, 2004), *Acinetobacter*, *Staphylococcus*, *Bacillus*

and *Sphingomonas*, and coliforms. Here we report on the taxonomic characterization of the orange-pigmented strain C42^T and the yellow-pigmented strain C52^T preliminarily identified as members of the genus *Sphingomonas*.

Strain C42^T was isolated from a treatment table after inoculation on PYE agar (1⁻¹: 3.0 g peptone from casein, 3.0 g yeast extract, 15.0 g agar; pH 7.2). Strain C52^T was isolated from a wipe in the treatment room after cultivation on PYE agar. Single colonies were visible after cultivation at 28 °C for 48 h.

The 16S rRNA gene was amplified and analysed as described previously (Zlamala *et al.*, 2002). The 16S rRNA gene sequences of strains C42^T and C52^T were continuous stretches of 1407 and 1408 nt, respectively, showing 94.7 % similarity to each other. Sequence comparisons (ungapped) performed using FASTA3 (Pearson & Lipman, 1988) indicated that the closest relatives of the orange-pigmented strain C42^T are *Sphingomonas aquatilis* KCTC 2881^T and *Sphingomonas melonis* DSM 14444^T (both showing 97.7 % sequence similarity). Only moderate sequence similarities were found with respect to the orange-pigmented species

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Sphingomonas abaci* C42^T and *Sphingomonas panni* C52^T are AJ575817 and AJ575818, respectively.

The fatty acid content, polar lipid profiles, protein patterns and genomic fingerprints of strains C42^T and C52^T are available as supplementary data in IJSEM Online.

(Busse *et al.*, 2003) *Sphingomonas faeni* MA-olki^T (96.2%), *Sphingomonas aurantiaca* MA101b^T (95.8%) and *Sphingomonas aerolata* NW12^T (95.7%) and to selected other species of the genus *Sphingomonas* (96.7–95.3%). Strain C52^T shared the greatest levels of sequence similarity with *Sphingomonas koreensis* KCTC 2882^T (97.2%), *S. aquatilis* KCTC 2881^T (97.1%) and *S. melonis* DSM 14444^T (97.0% sequence similarity). Sequence similarities with other species of the genus were below 96.8%. Phylogenetic analyses were performed using the PHYLIP package (Felsenstein, 1995). In all of the phylogenetic trees, strain C42^T grouped with *S. aquatilis* KCTC 2881^T and *S. melonis* DSM 14444^T, but the level of bootstrap support varied greatly according to the algorithm applied (maximum likelihood and maximum parsimony, <25%; neighbour-joining, 72%). Strain C52^T was always placed next to *S. koreensis* KCTC 2882^T with

high levels of bootstrap support (Fig. 1), confirming the results from sequence comparisons.

Physiological characterization was done as described previously (Kämpfer *et al.*, 1991). Temperature tolerance was examined on PYE agar, while NaCl tolerance was tested on PYE agar supplemented with different concentrations of NaCl. Growth on TSA, R2A and MacConkey agar was examined as well. Growth under microaerobic conditions (gas mixture 95% N₂, 5% CO₂, 1.5–2% remaining O₂) was examined on PYE agar. Obvious differences could be observed among the physiological properties of C42^T, C52^T and their close relatives, *S. aquatilis* KCTC 2881^T, *S. koreensis* KCTC 2882^T and *S. melonis* DSM 14444^T, which were analysed in parallel. The reactivity of our isolates was much lower than the reactivity of the reference strains. A high degree of similarity in terms of physiological characteristics (88%) was found for *S. aquatilis* KCTC 2881^T and *S. melonis* DSM 14444^T. Detailed results are listed in Table 1 and in the species descriptions below.

Chemotaxonomic analyses were performed as follows: respiratory quinones and polar lipids were determined according to Tindall (1990) and Altenburger *et al.* (1996), respectively, polyamines were analysed as described by Busse & Auling (1988) and Busse *et al.* (1997) and fatty acids were determined as described by Kämpfer *et al.* (1997). The results are given in the species descriptions and in Supplementary Table S1 (available in IJSEM Online).

The detection of quinone system Q-10 in C42^T and C52^T corresponded with species of the genus *Sphingomonas sensu stricto* and the family *Sphingomonadaceae* (Busse *et al.*, 1999; Kosako *et al.*, 2000). The polyamine patterns of the two strains showed the predominance of the compound sym-homospermidine, the key characteristic of *Sphingomonas sensu stricto* (Busse *et al.*, 1999; Takeuchi *et al.*, 2001), and minor amounts of spermidine. The polar lipid profiles of both strain C42^T and strain C52^T (see Supplementary Fig. S1, available in IJSEM Online) contained phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid and phosphatidylmethylethanolamine, which is in excellent agreement with the characteristics in the profiles of other species of the genus *Sphingomonas sensu stricto* (Busse *et al.*, 1999). However, their unique polar lipid profiles distinguished the two strains from each other and from other species of the genus *Sphingomonas sensu stricto* (Busse *et al.*, 1999).

The fatty acid profiles of strains C42^T and C52^T (Supplementary Table S1) showed the predominance of C_{18:1}ω7c and the relatively high levels of C_{16:0} that are shared with the majority of members of the 'Alpha-proteobacteria'. The presence of 2-hydroxy myristic acid (2-OH C_{14:0}) and the lack of 3-hydroxy fatty acids are important characteristics of members of the family *Sphingomonadaceae* (Busse *et al.*, 1999; Takeuchi & Hiraishi, 2001; Takeuchi *et al.*, 2001). However, our isolates differed from the reference strains both qualitatively and quantitatively with regard to certain acids.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) indicating the estimated phylogenetic position of strains C42^T and C52^T within the genus *Sphingomonas sensu stricto*. Sequences of reference species were obtained from the EMBL database, and the 16S rRNA gene sequence of *Escherichia coli* K-12 was selected as the outgroup. Sequences were aligned using CLUSTAL X (Thompson *et al.*, 1997) and manually edited to remove ambiguous nucleotides and gaps, using BioEdit (Hall, 1999). The tree was visualized using TreeView (Page, 1996). Numbers at nodes indicate levels of bootstrap support based on neighbour-joining analyses of 100 resampled datasets. Bar, 10 substitutions per 100 nt.

Table 1. Phenotypic characteristics of strains C42^T and C52^T and related type strains

Strains: 1, C42^T; 2, C52^T; 3, *S. melonis* DSM 14444^T; 4, *S. aquatilis* KCTC 2881^T; 5, *S. koreensis* KCTC 2882^T. +, Positive; (+), weakly positive; –, negative. Results were recorded after 72 h incubation at 30 °C. All strains grew under microaerobic conditions and were positive for hydrolysis of aesculin, *p*-nitrophenyl (pNP) β -D-glucopyranoside, pNP phenylphosphonate, 2-deoxythymidine-5'-pNP phosphate, L-alanine *p*-nitroanilide (pNA) and assimilation of L-malate. None of the strains was able to grow at 4 or 42 °C, on PYE agar supplemented with 5, 7.5 or 10% NaCl or on MacConkey agar. All strains were negative for acid production from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol, D-mannose, hydrolysis of L-proline pNA or assimilation of *N*-acetyl-D-galactosamine, α -D-melibiose, D-ribose, salicin, adonitol, i-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, itaconate, β -alanine, L-aspartate, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate.

Test	1	2	3	4	5
Pigmentation	Orange	Yellow	Yellow	Yellow	Yellow
Growth at 37 °C	–	+	–	–	+
Hydrolysis of:					
pNP β -D-galactopyranoside	+	+	–	–	–
pNP β -D-glucuronide	–	–	–	+	–
pNP α -D-glucopyranoside	–	+	+	+	+
pNP β -D-xylopyranoside	(+)	+	+	+	+
bis-pNP phosphate	+	+	(+)	–	+
pNP phosphorylcholine	–	+	+	+	+
L-Glutamate- γ -3-carboxy pNA	+	–	+	+	+
Assimilation of:					
<i>N</i> -Acetyl-D-glucosamine, D-fructose, L-rhamnose	–	–	+	–	+
Sucrose, D-xylose, <i>p</i> -arbutin, D-cellobiose, suberate, L-alanine	–	–	+	+	+
L-Arabinose	–	–	+	+	–
D-Galactose, DL-lactate	–	–	–	+	–
Gluconate	(+)	–	–	–	–
D-Glucose, mesaconate	–	–	+	–	(+)
D-Maltose, glutarate,	–	(+)	+	+	+
DL-3-hydroxybutyrate, L-proline					
D-Mannose, D-trehalose	–	(+)	+	–	(+)
Citrate	–	+	–	–	–
Fumarate	+	(+)	+	+	–
Oxoglutarate	(+)	+	–	–	–
Pyruvate	–	+	+	–	(+)
L-Histidine	–	–	–	–	+

Comparison of protein patterns after SDS-PAGE was performed with C42^T, C52^T, *S. aquatilis* KCTC 2881^T, *S. koreensis* KCTC 2882^T and *S. melonis* DSM 14444^T as described previously (Altenburger *et al.*, 1996). The protein patterns clearly distinguished both isolates from their closest relatives, *S. aquatilis* KCTC 2881^T, *S. koreensis* KCTC 2882^T and *S. melonis* DSM 14444^T. In contrast, almost identical protein patterns (Supplementary Fig. S2) were detected for *S. aquatilis* KCTC 2881^T (Lee *et al.*, 2001) and *S. melonis* DSM 14444^T (Buonauro *et al.*, 2002).

Genomic fingerprints (Supplementary Fig. S3) of C42^T, C52^T, *S. aquatilis* KCTC 2881^T, *S. koreensis* KCTC 2882^T and *S. melonis* DSM 14444^T obtained after ERIC-PCR (Wieser & Busse, 2000) clearly differentiated the five strains, but obvious similarities between *S. melonis* DSM 14444^T and *S. aquatilis* KCTC 2881^T, as might have been expected from their similar protein patterns, were not observed. Analysis of DNA relatedness (Ziemke *et al.*, 1998; Kämpfer *et al.*, 2003) between the pairs C42^T/*S. melonis* DSM 14444^T (19%, reciprocal 12%), C42^T/*S. aquatilis* KCTC 2881^T (17%,

reciprocal 17 %), *S. aquatilis* KCTC 2881^T/*S. melonis* DSM 14444^T (56 %, reciprocal 53 %), C52^T/*S. melonis* DSM 14444^T (23 %, reciprocal 17 %), C52^T/*S. aquatilis* KCTC 2881^T (18 %, reciprocal 15 %) and C52^T/*S. koreensis* KCTC 2882^T (23 %, reciprocal 24 %) did not reveal any relatedness at the species level.

The results of the analysis of 16S rRNA gene sequences, polyamine patterns, quinone system, fatty acids and polar lipids prove that strain C42^T and strain C52^T are both members of the genus *Sphingomonas sensu stricto*. The two isolates can be distinguished from each other on the basis of their 16S rRNA gene sequences and phenotypic traits (chemotaxonomically and physiologically) and from their close relatives by their genomic fingerprints, protein patterns, fatty acid composition and numerous physiological characteristics. On the basis of these results, we conclude that strains C42^T and C52^T represent two novel species of the genus *Sphingomonas sensu stricto*, for which we propose the names *Sphingomonas abaci* sp. nov. and *Sphingomonas panni* sp. nov., respectively.

Although *S. aquatilis* KCTC 2881^T and *S. melonis* DSM 14444^T show almost undistinguishable protein patterns and 16S rRNA gene sequences as well as relatively high degrees of similarity with respect to their fatty acid profiles and physiological traits, their DNA relatedness (~55 % similarity) does not suggest that they should be placed in a single species. However, this observation demonstrates that comparison of protein patterns is not suitable for the identification of strains as members of *S. melonis* or *S. aquatilis*.

Description of *Sphingomonas abaci* sp. nov.

Sphingomonas abaci (a'ba.ci. L. gen. n. *abaci* of/from a table, referring to the fact that the type strain was isolated from a treatment table).

Cells are Gram-negative (by staining and in the KOH test), non-spore-forming rods with rounded poles and are 0.4–0.5 × 1.5–3.0 µm in size. Cells occur singly or in pairs (rarely). Oxidase-negative and catalase-positive; growth occurs under aerobic and microaerobic conditions but not under anaerobic conditions. No motility is observed under the light microscope. Good growth occurs on PYE agar, TSA, 1 % (w/v) NaCl and at temperatures between 15 and 28 °C on PYE agar. No growth occurs on MacConkey agar or at 4, 37 and 42 °C on PYE agar. On PYE agar, orange, shiny, circular, leathery, dry colonies approximately 0.5 mm in diameter form within 48 h. After 5 days incubation, colonies on PYE agar reach diameters of approximately 2 mm. The quinone system of the type strain consists of Q-10 (86 %), Q-9 (9 %) and Q-8 (5 %). The polyamine pattern primarily consists of *sym*-homospermidine [48.1 µmol (g dry weight)⁻¹] with moderate amounts of spermidine [12.0 µmol (g dry weight)⁻¹]. The predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and sphingoglycolipid. Additionally, moderate amounts of phosphatidylcholine, three unidentified glycolipids and an

unknown polar lipid, small amounts of phosphatidylidimethylethanolamine, phosphatidylmonomethylethanolamine and three unknown phospholipids are present. The fatty acids comprise C_{18:1}ω7c (64.8 %), 2-OH C_{14:0} (12.9 %), C_{16:0} (12.4 %), summed feature 3 (C_{16:1}ω7c and/or 2-OH C_{15:0} iso) (8.3 %) and C_{17:1}ω6c (1.6 %). Acid production from sugars, carbon-source utilization and hydrolysis of chromogenic substrates are listed in Table 1.

The type strain, C42^T (= LMG 21978^T = DSM 15867^T), was isolated from the treatment table in the Medical Clinic for Small Animals, University for Veterinary Medicine, Vienna, Austria.

Description of *Sphingomonas panni* sp. nov.

Sphingomonas panni (pan'ni. L. gen. n. *panni* of/from a piece of cloth, or by extension a wipe, referring to the fact that the type strain was isolated from a wipe).

Cells are Gram-negative (by staining and in the KOH test), non-spore-forming rods with rounded poles and are approximately 0.5–0.7 × 1.2–2.0 µm in size. Cells occur singly or in pairs (rarely). Oxidase-negative and catalase-positive; growth occurs under aerobic and microaerobic conditions but not under anaerobic conditions. No motility is observed under the light microscope. Good growth occurs on TSA, R2A, at 1 % (w/v) NaCl and at temperatures between 15 and 37 °C on PYE agar. No growth occurs on MacConkey agar or at 4 and 42 °C on PYE agar. Yellow, shiny, circular colonies approximately 0.3 mm in diameter form within 48 h on PYE agar. After 5 days incubation, colonies reach diameters of about 1.5 mm. The quinone system of the type strain consists of Q-10 (95 %), Q-9 (3 %) and Q-8 (1 %). The polyamine pattern primarily consists of *sym*-homospermidine [36.0 µmol (g dry weight)⁻¹] with minor amounts of spermidine [1.3 µmol (g dry weight)⁻¹]. The predominant polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and sphingoglycolipid. Additionally, small amounts of phosphatidylidimethylethanolamine, diphosphatidylglycerol and an unidentified phospholipid are present. The fatty acids comprise C_{18:1}ω7c (51.3 %), C_{16:0} (17.8 %), summed feature 3 (C_{16:1}ω7c and/or 2-OH C_{15:0} iso) (17.1 %), 2-OH C_{14:0} (5.3 %), C_{17:1}ω6c (3.6 %), C_{16:1}ω5c (2.1 %), C_{15:0} (1.1 %), C_{14:0} (1.1 %) and C_{18:1}ω5c (0.7 %). Acid production from sugars, carbon-source utilization and hydrolysis of chromogenic substrates are listed in Table 1.

The type strain, C52^T (= LMG 21979^T = DSM 15761^T), was isolated from a wipe in the Medical Clinic for Small Animals, University of Veterinary Medicine, Vienna, Austria.

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