Sphingomonas aurantiaca sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas*

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Seven psychrotolerant, Gram-negative bacterial strains, five dust- and airborne isolates (MA101b^T, MA306a, MA405/90, MA-olki^T and NW12^T) and two from the Antarctic (Ant 20 and M3C203B-B), were subjected to a polyphasic characterization to determine their taxonomic position. High 16S rDNA sequences similarities (99·3–100·0 %) demonstrated that they were closely related to each other. Phylogenetic evaluation of their 16S rDNA sequences revealed that they are members of the genus *Sphingomonas sensu stricto*, encompassing a separate branch within this genus. They shared 94·4–96·6 % 16S rDNA sequence similarity with species of this genus. All *Sphingomonas*-specific signature nucleotides were also detected. The presence of the major ubiquinone Q-10, *sym*-homospermidine as the predominant polyamine, *Sphingomonadaceae*-specific sphingoglycolipid in the polar lipid patterns and a fatty acid profile containing $C_{14:0}$ 2-OH and lacking 3-OH fatty acids were in agreement with identification of these strains as members of the genus *Sphingomonas sensu stricto*. Results from DNA–DNA hybridizations and comparison of protein patterns indicated that the seven strains are members of three distinct species. One species is represented by strains MA101b^T, MA306a and MA405/90, the second by strains NW12^T, Ant 20 and M3C203B-B and the third by one strain, MA-olki^T. Their distinction at the species level was also supported by results of biochemical characterization and partly supported by riboprints and genomic fingerprints. On the basis of these results, three novel species of the genus *Sphingomonas* are proposed: *Sphingomonas aurantiaca* sp. nov., consisting of strains MA101b^T (=DSM 14748^T=LMG 21377^T), MA306a and MA405/90 (=DSM 14749=LMG 21378), *Sphingomonas faeni* sp. nov. MA-olki^T (=DSM 14747^T=LMG 21379^T) and *Sphingomonas aerolata* sp. nov., represented by strains NW12^T (=DSM 14746^T=LMG 21376^T), Ant 20 (=ICMP 13599) and M3C203B-B (=SMCC M3C203B-B).

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The EMBL accession numbers for the 16S rDNA sequences of *Sphingomonas aurantiaca* strains MA101b^T, MA306a and MA405/90, *Sphingomonas faeni* MA-olki^T and *Sphingomonas aerolata* NW12^T are respectively AJ429236–AJ429240.

Riboprints of the novel isolates and the results of TLC analysis of the polar lipids of a representative strain are available as supplementary material in IJSEM Online.

Airborne bacteria are of special interest because they have to survive in an unfavourable environment. They are able to withstand increased radiation and desiccation compared with the majority of bacteria, which do not survive for more than a few seconds when suspended in the air (Stetzenbach, 1992). During studies on dustborne bacteria in Finland (Andersson *et al.*, 1999) and airborne bacteria in England (Brimblecombe *et al.*, 1999), five orange-pigmented bacterial strains were isolated. Preliminary analyses of the 16S rDNA sequences of the five strains revealed their affiliation with the genus *Sphingomonas sensu stricto* as defined by Takeuchi *et al.* (2001). The ability to survive when suspended in the air has not been reported for members of the genus *Sphingomonas sensu stricto* and, what is more, orange pigmentation is not given in the description of the genus *Sphingomonas sensu stricto*, which encompasses only yellow- or off-white-pigmented species (Takeuchi *et al.*, 2001).

The genus *Sphingomonas*, originally described by Yabuuchi *et al.* (1990), has recently been dissected into four genera (Takeuchi *et al.*, 2001). The genus name *Sphingomonas* was retained for those species that are most closely related to *Sphingomonas paucimobilis*, the type species of the genus (cluster I according to Takeuchi *et al.*, 2001). Species of *Sphingomonas sensu stricto* can be distinguished from species of other genera of the family *Sphingomonadaceae* by the presence of *sym*-homospermidine as the predominant compound in the polyamine patterns, several signature nucleotides in the 16S rDNA sequences and a combination of other phenotypic markers. The presence of *sym*-homospermidine as a distinguishing character within the family *Sphingomonadaceae* is not considered in taxonomic considerations by some scientists (Yabuuchi *et al.*, 1990, 2002), although it reflects the separate phylogenetic position of the genus *Sphingomonas sensu stricto*. Thus, we strongly support the proposal to retain the genus name *Sphingomonas* only for the members of cluster I, and the nomenclature of Takeuchi *et al.* (2001) is used in this report.

Here, classification of these five isolates and two Antarctic *Sphingomonas* strains (Aislabie *et al.*, 2000; Christner *et al.*, 2000) and their description as three novel species of the genus *Sphingomonas* are reported.

Isolation and cultivation

Strains MA101b^T, MA306a and MA405/90 were isolated in Spring 1990 and strain MA-olki^T was isolated in December 1998 from cow-barn air when bales of hay and straw were broken open (Andersson *et al.*, 1999). Isolation was done on TSA at 16–18 °C. Strain NW12^T was isolated on CasMM agar (Altenburger *et al.*, 1996) in December 1996 during a campaign for collection of airborne micro-organisms in the Sainsbury Centre for Visual Arts in Norwich, UK (Brimblecombe *et al.*, 1999) and was subcultivated at room temperature on PYES agar (Buczolits *et al.*, 2002).

16S rDNA sequence analysis

16S rDNA was amplified and analysed as described previously (Abraham *et al.*, 1999). Sequence comparisons (ungapped) using FASTA3 (Pearson & Lipman, 1988) revealed: 99·7 % similarity between strain MA-olki^T and the three strains MA101b^T, MA306a and MA405/90; 99·6 % similarity between strain NW12^T and strains MA101b^T, MA306a and MA405/90; and 99·3 % similarity between strains NW12^T and MA-olki^T. Highest similarities were found with sequences of established species of the genus *Sphingomonas sensu stricto* including *Sphingomonas aquatilis* KCTC 2881^T (96·4–96·6 %), *Sphingomonas mali* IFO 15500^T (95·7–96·1 %), *Sphingomonas pruni* IFO 15498^T (95·6–96·0 %), *Sphingomonas asaccharolytica* IFO 15499^T (95·5–96·0 %), *Sphingomonas adhaesiva* GIFU 11458^T (95·4–96·0 %) and *Sphingomonas echinoides* DSM 1805^T (95·9–96·0 %). These results clearly demonstrate that the five isolates are members of the genus *Sphingomonas sensu stricto*. Assignment to the genus *Sphingomonas sensu stricto* was also confirmed by the presence in the sequences of the five isolates of all signature nucleotides that characterize the genus (Takeuchi *et al.*, 2001). However, the 16S rDNA sequences of the five isolates shared highest similarities with sequences of *Sphingomonas* strain Ant 20, isolated from hydrocarbon-contaminated soils around Scott Base, Antarctica (Aislabie *et al.*, 2000), *Sphingomonas* strain M3C203B-B, isolated from ice of Taylor Dome, Antarctica (Christner *et al.*, 2000, 2001), and *Sphingomonas* strain V21 (accession no. AF324199), isolated from Lake Vostok accretion ice, Antarctica (Christner *et al.*, 2001). Both strains Ant 20 and M3C203B-B showed 99·9 % sequence similarity to strain NW12^T, 99·6 % to strains MA101b^T, MA306a and MA405/90 and 99·3 % to strain MA-olki^T. The sequence of strain V21 was 98·2 % similar to those of strains MA101b^T, MA306a, MA405/90 and MA-olki^T and 97·3 % similar to that of strain NW12^T. Like our air- and dustborne isolates, strain Ant 20 forms orange-pigmented colonies (Aislabie *et al.*, 2000), as does strain M3C203B-B, whereas V21 is yellow-pigmented (unpublished results). Orange pigmentation is rare among members of the family *Sphingomonadaceae* and this trait, in addition to psychrotolerance and extraordinarily high sequence similarities, indicated a relationship between strains Ant 20, M3C203B-B and NW12^T at the species level. Thus, strains Ant 20 and M3C203B-B were included in our study. Strain V21 was not included since it is not orange-pigmented and its 16S rDNA sequence similarity to the group of orange-pigmented strains was significantly lower than the similarities between strains within this group.

Nucleotide sequences were aligned with reference 16S rDNA sequences using evolutionarily conserved primary sequence and secondary structure (Gutell *et al.*, 1985) as references. Evolutionary distances (Jukes & Cantor, 1969) were calculated from nearly complete sequence-pair dissimilarities using only homologous, unambiguously determined nucleotide positions. Bootstrap analysis and phylogenetic trees were constructed using programs

implemented in the PHYLIP package (Felsenstein, 1993). Phylogenetic analysis of nearly full-length 16S rDNA sequences of the five isolates confirmed that they are closely related to each other, as already indicated from sequence similarities, and that they are members of the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001), forming a distinct phylogenetic lineage (Fig. 1).

Physiological and biochemical characteristics

The seven isolates, including Ant 20 and M3C203B-B, grew on TSA at temperatures between 4 and 28 °C, but did not grow at 37 °C. Standard bacteriological and biochemical characterization was carried out as described previously (Denner *et al.*, 2001; Kämpfer *et al.*, 1991), except that the tests were incubated at room temperature for 7 days. Additionally, the strains were studied using API 20NE and API ZYM galleries (bioMérieux), according to the instructions of the manufacturer. Biochemical characteristics were studied as described by Kämpfer *et al.* (1991). For 103 characteristics tested (Table 1), strains MA101b^T, MA306a and MA405/90 were homogeneous and differed from each other in not more than five traits. Another homogeneous group was formed by NW12^T, Ant 20 and M3C203B-B, which again differed from each other in not more than five characteristics. These two groups could be distinguished from each other by five traits and from strain MA-olki^T by seven and ten traits, respectively.

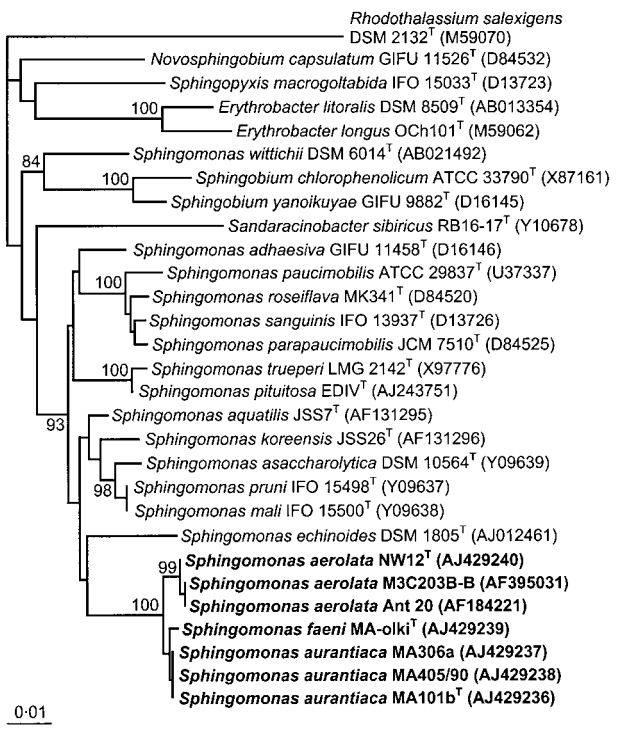

Fig. 1. Estimated 16S rDNA phylogenetic relationships among strains MA-olki^T, NW12^T, MA101b^T, MA306a and MA405/90 and related species of the genus *Sphingomonas sensu stricto*. Bar, 1 substitution per 100 nucleotide positions. Dataset was resampled 100 times to obtain bootstrap values of confidence. Branching edges for bootstrap values >70 % are shown.

Genomic analyses

Determination of DNA relatedness between the five strains was done as described by Ziemke *et al.* (1998). The results of DNA–DNA hybridizations (Table 2) demonstrate that strains MA101b^T, MA306a and MA405/90 are members of a single species, that strains NW12^T, Ant 20 and M3C203B-B represent another species and that strain MA-olki^T represents a third species. Analysis of genomic DNA using ERIC-PCR (enterobacterial repetitive intergenic consensus PCR) and BOX-PCR (BOX element PCR) (Wieser & Busse, 2000) revealed unique band patterns (Fig. 2) for strains MA-olki^T, NW12^T and MA405/90, whereas genomic fingerprints from strains MA101b^T and MA306a were almost indistinguishable. This observation indicates that the latter two strains are clonally related. MA405/90 shared one characteristic band with MA101b^T and MA306a in the ERIC-PCR fingerprint (at approx. 400 bp) and BOX-PCR fingerprint (at approx. 300 bp), suggesting moderate relatedness. No significant similarities were observed between the ERIC- and BOX-PCR-generated genomic fingerprints of strains NW12^T, Ant 20 and M3C203B-B (results not shown). Riboprints were analysed as described previously (Busse *et al.*, 2000). High degrees of similarity were observed between the riboprints of strains MA101b^T, MA306a and MA405/90 (>63 %) and strains Ant 20 and M3C203B-B (76 %). No significant similarities (<54 %) were detected between other riboprints (see Supplementary Fig. A in IJSEM Online). The G+C contents of the genomic DNAs were determined from HPLC analyses of nucleosides (Kaneko *et al.*, 1986; Busse *et al.*, 2002). The G+C contents of strains MA-olki^T, NW12^T and MA101b^T were respectively 63·1, 65·4 and 64·7 mol%.

Chemotaxonomic characterization

Polyamines were analysed as described previously (Busse & Auling, 1988; Busse *et al.*, 1997). The polyamine patterns of our five isolates and the two Antarctic strains contained predominantly *sym*-homospermidine. Other polyamines were only detected in trace amounts. This polyamine pattern is a characteristic feature of the genus *Sphingomonas sensu stricto* as defined previously (Busse *et al.*, 1999; Takeuchi *et al.*, 2001). The quinone systems of the five strains, which were determined by HPLC (Tindall, 1990; Altenburger *et al.*, 1996), consisted predominantly of ubiquinone Q-10 (82–96 %), with small amounts of Q-9 (3–12 %) and Q-8 (1–6 %). This is in accordance with results for other members of the *Sphingomonadaceae* (Busse *et al.*, 1999).

Polar lipid profiles were analysed according to the method of Ventosa *et al.* (1993). For comparability with results from our previous work (Busse *et al.*, 1999), the same designations for unknown lipids that display similar

Table 1. Biochemical characteristics of orange-pigmented *Sphingomonas* strains

Taxa: 1, *Sphingomonas faeni* sp. nov. MA-olki^T; 2, *Sphingomonas aerolata* sp. nov. (3 strains); 3, *Sphingomonas aurantiaca* sp. nov. (3 strains). +, Positive; (+), weakly positive; −, negative; V, variable. All strains were oxidase- and catalase-positive and assimilated *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, sucrose, salicin, D-trehalose, D-xylose, maltitol, fumarate, DL-3-hyroxybutyrate, suberate and L-alanine. All strains assimilated acetate, adipate and oxoglutarate, although weakly positive results were observed for strains Ant 20 and M3C203B-B. All strains hydrolysed aesculin, *p*-nitrophenyl (pNP) β-D-glucopyranoside, 2-deoxythymidine-5′-pNP phosphate, L-alanine *p*-nitroanilide (pNA) and L-glutamate-γ-3-carboxy pNA. In API ZYM test, all strains were positive for alkaline phosphatase, esterase (C4), esterase (C8), leucine arylamidase, acidic phosphatase and β-glucosidase and negative for trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. All strains were negative in the O/F and Simmons' citrate tests, did not reduce nitrate and did not produce indole or H_2S. None of the seven strains assimilated D-ribose, adonitol, D-mannitol, D-sorbitol, putrescine, *cis*-aconitate, 4-aminobutyrate, itaconate, mesaconate, β-alanine, L-histidine, L-serine, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate or phenylacetate or hydrolysed starch, casein, gelatin, pNP β-D-galactopyranoside, pNP phenylphosphonate or pNP phosphorylcholine.

Character	1	2	3
Assimilation of:			
L-Rhamnose, DL-lactate, L-aspartate	+	−	−
α-D-Melibiose, citrate	+	V[a⋆]	+
i-Inositol	−	−	V[e]
Propionate	+	−	V[f]
trans-Aconitate	−	−	V[f]
Azelate, L-leucine	+	−	+
Glutarate, pyruvate	+	V[b]	+
L-Malate	−	V[c]	+
L-Proline	−	−	+
4-Hydroxybenzoate	−	−	V[g]
Hydrolysis of:			
pNP α-D-glucopyranoside	−	+	V[h]
bis-pNP phosphate	−	+	+
L-Proline pNA	+	−	V[f]
pNP β-D-glucuronide	−	+	−
DNA	(+)	(+)	V[j]
Enzymic activities (API ZYM):			
Valine arylamidase	+	+	−
α-Glucosidase	(+)	+	V[g]
β-Galactosidase	(+)	+	(+)
Naphthol-AS-BI-phosphohydrolase	(+)	+[d]	(+)
Lipase (C14)	−	−	V[k]
Cystine arylamidase	+	V[a]	V[g]

*Strain-dependent results are indicated by: *a*, only NW12^T positive; *b*, Ant 20 and M3C203B-B weakly positive and NW12^T negative; *c*, NW12^T positive, M3C203B-B weakly positive and Ant 20 negative; *d*, Ant 20 and M3C203B-B weakly positive; *e*, only MA405/90 positive; *f*, only MA405/90 negative; *g*, only MA101b^T negative; *h*, only MA306a negative; *j*, MA306a weakly positive and MA101b^T negative; *k*, only MA101b^T positive.

chromatographic and staining behaviour are used here. The profiles of strains MA-olki^T, MA101b^T and MA405/90 were almost identical. They were characterized by the presence of phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline, diphosphatidyl glycerol and sphingoglycolipid as the predominant lipids, moderate amounts of phosphatidyl dimethylethanolamine and two unidentified phospholipids (PL3, PL6) and traces of phosphatidyl monomethylethanolamine. Additionally, MA-olki^T contained minor amounts of an unidentified aminophospholipid (APL1). NW12^T could be distinguished from the strains mentioned above by the presence of an unidentified

Table 2. DNA–DNA relatedness between orange-pigmented *Sphingomonas* strains

Source of unlabelled DNA	Relatedness (%) with labelled DNA from:			
	MA306a	MA-olki^T	MA405/90	NW12^T
MA306a	100	60	100	ND
MA 101b^T	118	52	91	ND
MA 405	81	51	100	ND
MA-olki^T	57	100	61	ND
NW12^T	48	35	35	100
Ant 20	ND	ND	ND	71*
M3C203B-B	ND	ND	ND	107†
Sphingomonas paucimobilis NCTC 11030^T	29	29	29	ND
Pooled SD	4·1	4·9	5·1	

*Mean of three measurements (relatedness values of 62, 68 and 84 %).
†Mean of three measurements (relatedness values of 103, 109 and 109 %).
ND, Not determined.

phospholipid (PL5), an aminophospholipid (APL2) and three glycolipids (GL1, GL4, GL5) (see Supplementary Fig. B in IJSEM Online). The polar lipid profile of strain M3C203B-B was almost identical to that of NW12^T. The polar lipid profile of strain Ant 20 resembled those of NW12^T and M3C203B-B, but it could be distinguished from these two strains by the presence of small amounts of three unidentified, additional phospholipids and an aminophospholipid. These polar lipid profiles clearly distinguished the strains studied here from members of the *Sphingomonadaceae* (Busse *et al.*, 1999), including *Blastomonas natatoria* DSM 3183^T, a strain that turns from yellow to orange as cultures age.

During analysis of polar lipids, it was revealed that the orange pigmentation of the isolates resulted from the presence of different orange compounds. Analysis of acetone-extracted pigments (Denner *et al.*, 2001) of the seven strains displayed spectra that were indicative only for yellow pigments. The spectra obtained were similar, but not identical, to those reported for other sphingomonads, including strains of *Sphingomonas paucimobilis*, *Sphingomonas trueperi* (Jenkins *et al.*, 1979), *Sphingomonas pituitosa* DSM 13101^T (Denner *et al.*, 2001), *Novosphingobium capsulatum* LMG 2830^T (Bally *et al.*, 1990), *Sphingomonas xenophaga* DSM 6383^T (Stolz *et al.*, 2000), which can be considered to be a member of the genus *Sphingobium* (Takeuchi *et al.*, 2001), and *Erythrobacter citreus* DSM 14432^T (Denner *et al.*, 2002). The spectral characteristics of the extracted pigments of the strains were as follows: MA101b^T, MA306a, Ant 20 and M3C203B-B, λ_{max} at 458 and 478 nm; MA405/90, λ_{max} at 457 and 478 nm; MA-olki^T, λ_{max} at 458 and 474 nm; and NW12^T, λ_{max} at 458 and 476–477 nm. Several spectra showed slight inflexions at approximately 425 nm.

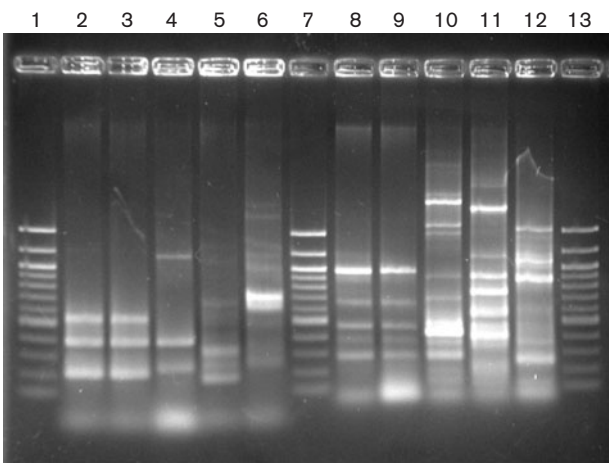

Fig. 2. Genomic fingerprints of the five orange-pigmented isolates generated by ERIC-PCR (lanes 2–6) and BOX-PCR (8–12). Lanes: 1, 7 and 13, 100 bp ladder; 2 and 8, MA101b^T; 3 and 9, MA306a; 4 and 10, MA405/90; 5 and 11, MA-olki^T; 6 and 12, NW12^T.

Fatty acids were analysed from biomass that was grown for 72 h on TSA at 28 °C as described by Kämpfer *et al.* (1997). The seven strains displayed almost identical fatty acid profiles, including the presence of $C_{14:0\ 2\text{-}OH}$ and the absence of any 3-OH fatty acids (Table 3). These profiles are in excellent agreement with those of representatives of the genus (Kämpfer *et al.*, 1997; Busse *et al.*, 1999; Denner *et al.*, 2001) and their assignment to the genus *Sphingomonas sensu stricto*.

Almost identical protein band patterns obtained after SDS-PAGE (Altenburger *et al.*, 1996) indicated that strains MA101b^T, MA306a and MA405/90 are strains of a single species, NW12^T, Ant 20 and M3C203B-B are members of another species and MA-olki^T represents a third species (results not shown). These observations are in agreement with results from 16S rDNA sequence comparisons, DNA–DNA hybridizations and polar lipid profiles.

Table 3. Fatty acid compositions of *Sphingomonas faeni*, *Sphingomonas aerolata* and *Sphingomonas aurantiaca*

Species: 1, *Sphingomonas faeni* sp. nov. MA-olki^T; 2, *Sphingomonas aerolata* sp. nov. strains NW12^T, Ant 20 and M3C203B-B; 3, *Sphingomonas aurantiaca* sp. nov. strains MA101b^T, MA306a and MA405/90. Values are percentages of total fatty acids. The position of the double bond can be located by counting from the methyl (ω) end of the carbon chain. Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contains one or more of $C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH. Summed feature 7 contains one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$ (*cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively).

Fatty acid	1	2	3
$C_{14:0}$	2·0	1·6–1·9	2·1–3·0
$C_{12:0}$ 2-OH	0·6	0·3–0·6	0·4
$C_{14:0}$ 2-OH	9·7	8·7–14·1	10·0–10·6
$C_{15:0}$		0·0–0·5	0·0–0·8
Summed feature 4	29·2	24·6–26·8	25·7–28·0
$C_{16:1}\omega5c$	1·6	1·2–2·0	1·6–1·8
$C_{16:0}$	11·2	12·0–15·8	12·2–15·6
$C_{17:0}$ cyclo		0·0–0·4	
$C_{17:1}\omega6c$	2·6	0·0–1·7	0·8–3·0
$C_{17:1}\omega8c$			0·0–0·4
$C_{16:1}$ 2-OH		0·0–0·5	
$C_{17:0}$			0·0–0·2
Summed feature 7	41·4	38·5–46·0	40·0–40·5
$C_{18:1}\omega5c$	1·0	0·6–0·8	0·6–1·0
$C_{18:0}$			0·0–0·3
$C_{19:0}$ cyclo $\omega8c$	0·8		0·4–0·7
11-methyl $C_{18:1}\omega7c$			0·0–3·5

Taxonomic considerations

Polyphasic classification of the dustborne strains MA101b^T, MA306a, MA405/90 and MA-olki^T, the airborne strain NW12^T and the two Antarctic strains Ant 20 and M3C203B-B demonstrated that they are closely related to each other and members of the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001). Results from 16S rDNA analyses, DNA–DNA hybridizations, riboprint analyses, ERIC- and BOX-PCR, polar lipid profiles and protein patterns separated strains MA101b^T, MA306a and MA405/90 from the other four strains. 16S rDNA analyses, DNA similarities and protein patterns suggest that strains NW12^T, M3C203B-B and Ant 20 form a second group that is heterogeneous in terms of genomic fingerprints and partly heterogeneous in polar lipid profiles and riboprint patterns. Strain MA-olki^T can be distinguished from the other strains by its 16S rDNA sequence, DNA similarity data, genomic fingerprints, riboprint patterns, protein patterns and polar lipid profiles. Biochemical traits support the distinction of the seven strains into three groups. Based on these results, it is concluded that our five isolates and the two Antarctic strains represent three novel species of the genus *Sphingomonas sensu stricto*. Therefore, the names *Sphingomonas aerolata* sp. nov. (for strains NW12^T, Ant 20 and M3C203B-B), *Sphingomonas faeni* sp. nov. (for strain MA-olki^T) and *Sphingomonas aurantiaca* sp. nov. (for strains MA101b^T, MA306a and MA405/90) are proposed.

The affiliation of these species, which differ from the genus description in their pigmentation and cell sizes, to the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001), as well as the lack of information concerning polar lipid compositions within the genus (Busse *et al.*, 1999), suggests that the description of the genus *Sphingomonas* should be emended.

Emended description of the genus *Sphingomonas* Yabuuchi *et al.* 1990 emend. Takeuchi *et al.* 2001

Cells are 0·3–0·8 × 1·0–2·7 μm. Colonies are off-white-, yellow- or orange-pigmented. The polar lipid profiles contain the following, in addition to sphingoglycolipid: phosphatidyl glycerol as predominant lipid, moderate to large amounts of phosphatidyl ethanolamine, diphosphatidyl glycerol, phosphatidyl dimethylethanolamine and phosphatidyl choline, varying amounts of phosphatidyl monomethylethanolamine and varying numbers of unidentified polar lipids (Busse *et al.*, 1999). Other characteristics of the genus are those given by Takeuchi *et al.* (2001).

Description of *Sphingomonas aerolata* sp. nov.

Sphingomonas aerolata (ae.ro.la′ta. Gr. fem. n. *aer* air; L. part. adj. *lata* carried; N.L. part. adj. *aerolata* airborne).

Cells are small rods, 0·6–0·8 × 1·5–2·6 μm. Growth is observed on Czapek–Dox, R2A, CasMM, PYES and TSA, but not on MacConkey agar. Cells occur singly or sometimes in short chains. Cells grow at 4–28 °C, but not at 37 °C. Gram-negative as determined by Gram staining, KOH and aminopeptidase tests. Motile. Endospores not observed. Colonies are circular, slightly convex, opaque and orange-pigmented. Aerobic. Catalase- and oxidase-positive. Nitrate is not reduced. Strain Ant 20 mineralizes phenanthrene and 1-methyl naphthalene (Aislabie *et al.*, 2000). Other physiological and biochemical traits are shown in Table 1. Polar lipid profile consists of phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline, diphosphatidyl glycerol, sphingoglycolipid and unknown glycolipid 1 as the predominant lipids, moderate amounts of phosphatidyl dimethylethanolamine, three unidentified phospholipids and two glycolipids and traces of phosphatidyl monomethylethanolamine and an unidentified aminophospholipid. Three unidentified phospholipids and an aminophospholipid may also be present. Predominant compounds in the fatty acid profile are summed feature 7 ($C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$), summed feature 4 ($C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH), $C_{16:0}$ and $C_{14:0}$ 2-OH. Major quinone is ubiquinone Q-10. Predominant polyamine is *sym*-homospermidine. Acetone-soluble pigment is

characterized by λ_{max} at 458 and 476–478 nm. The G+C content of genomic DNA of the type strain is 65·4 mol%.

The type strain, NW12^T (=DSM 14746^T=LMG 21376^T), was isolated from air in the Sainsbury Centre for Visual Arts, Norwich, UK. Strain Ant 20 (=NCMP 13599) was isolated from hydrocarbon-contaminated soils around Scott Base, Antarctica (Aislabie *et al.*, 2000), and strain M3C203B-B (=SMCC M3C203B-B) was isolated from 4200-year-old ice of Taylor Dome, Antarctica (Christner *et al.*, 2000, 2001).

Description of *Sphingomonas faeni* sp. nov.

Sphingomonas faeni (fae′ni. L. gen. n. *faeni* of hay).

Cells are small rods, 0·6–0·8 × 2·0–2·6 μm. Growth is observed on Czapek–Dox, R2A, CasMM, PYES and TSA, but not on MacConkey agar. Cells occur singly or sometimes in short chains. Cells grow on TSA at 4–28 °C, but not at 37 °C. Gram-negative as determined by Gram staining, KOH and aminopeptidase tests. Motile. Endospores not observed. Colonies are circular, slightly convex, opaque and orange-pigmented. Aerobic. Catalase- and oxidase-positive. Nitrate is not reduced. Other physiological and biochemical traits are shown in Table 1. Polar lipid profile consists of phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline, diphosphatidyl glycerol and sphingoglycolipid as the predominant lipids, moderate amounts of phosphatidyl dimethylethanolamine and two unidentified phospholipids and traces of phosphatidyl monomethylethanolamine and an unidentified aminophospholipid. Predominant compounds in the fatty acid profile are summed feature 7 ($C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$), summed feature 4 ($C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH), $C_{16:0}$ and $C_{14:0}$ 2-OH. Major quinone is ubiquinone Q-10. Predominant polyamine is *sym*-homospermidine. Acetone-soluble pigment is characterized by λ_{max} at 457–458 and 478 nm. The G+C content of genomic DNA of the type strain is 63·1 mol%.

The type strain, MA-olki^T (=DSM 14747^T=LMG 21379^T), was isolated from whirled-up dust in a cow barn, Finland.

Description of *Sphingomonas aurantiaca* sp. nov.

Sphingomonas aurantiaca (au.ran.ti.a′ca. M.L. fem. adj. *aurantiaca* orange-coloured).

Cells are small rods, 0·6–0·8 × 1·0–2·7 μm. Growth is observed on Czapek–Dox, R2A, CasMM, PYES and TSA, but not on MacConkey agar. Cells occur singly or sometimes in short chains. Cells grow on TSA at 4–28 °C, but not at 37 °C. Gram-negative as determined by Gram staining, KOH and aminopeptidase tests. Motile. Endospores not observed. Colonies are circular, slightly convex, opaque and orange-pigmented. Aerobic. Catalase- and oxidase-positive. Nitrate is not reduced. Other physiological and biochemical traits are shown in Table 1. Polar lipid profile consists of phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline, diphosphatidyl glycerol and sphingoglycolipid as the predominant lipids, moderate amounts of phosphatidyl dimethylethanolamine and two unidentified phospholipids and traces of phosphatidyl monomethylethanolamine. Predominant compounds in the fatty acid profile are summed feature 7 ($C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$), summed feature 4 ($C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH), $C_{16:0}$ and $C_{14:0}$ 2-OH. Major quinone is ubiquinone Q-10. Predominant polyamine is *sym*-homospermidine. Acetone-soluble pigment is characterized by λ_{max} at 458 and 476–477 nm. The G+C content of genomic DNA of the type strain is 64·7 mol%.

The type strain, MA101b^T (=DSM 14748^T=LMG 21377^T), and strains MA405/90 (=DSM 14749=LMG 21378) and MA306a were isolated from whirled-up dust in a cow barn, Finland.

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