Roseomonas aerilata sp. nov., isolated from an air sample

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A polyphasic study was carried out to clarify the taxonomic position of a pink-coloured, Gram-negative bacterium isolated from air in the Suwon region of Korea. The novel strain, 5420S-30^T, grew in the temperature range of 5–35 °C (optimum 30 °C) and pH range of 5–9 (optimum pH 6–7). Growth did not occur in the presence of as little as 1 % (w/v) NaCl. It contained Q-10 as the predominant ubiquinone. The major fatty acids were C_{18:1}ω7c, summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH) and C_{16:0} (together representing 77.7 % of the total). The DNA G+C content was 72.1 mol%. The 16S rRNA gene sequence similarities between strain 5420S-30^T and members of the genus *Roseomonas* were in the range 91.6–95.1 %, with the highest sequence similarities (95.1 and 94.5 %, respectively) to *Roseomonas aquatica* TR53^T and *Roseomonas gilardii* ATCC 49956^T. On the basis of its phenotypic and genotypic characteristics, strain 5420S-30^T represents a novel species of the genus *Roseomonas*, for which the name *Roseomonas aerilata* sp. nov. is proposed, with 5420S-30^T (=KACC 12521^T =DSM 19363^T) as the type strain.

The genus *Roseomonas* was first proposed for pink-coloured, Gram-negative, coccoid-shaped isolates that were pathogenic to humans (Rihs *et al.*, 1993). To date, *Roseomonas gilardii* (Rihs *et al.*, 1993), *Roseomonas cervicalis* (Rihs *et al.*, 1993), *Roseomonas mucosa* (Han *et al.*, 2003), *Roseomonas lacus* (Jiang *et al.*, 2006) and *Roseomonas aquatica* (Gallego *et al.*, 2006) have been described. *Roseomonas fauriae* was combined with *Azospirillum brasilense* as a later synonym (Helsel *et al.*, 2006).

Air (approx. 50 l) was sampled at an urban site in Suwon city, Republic of Korea (37° 16' 46" N 126° 59' 10" E), using an MAS-100 air sampler (single-stage multiple-hole impactor; Merck) containing a Petri dish with R2A agar (BBL) amended with 200 µg cycloheximide ml⁻¹ (Sigma). After sampling, the plate was incubated at 30 °C for 5 days and a pink-coloured bacterial strain, 5420S-30^T, was recovered. Routine cultivation was conducted at 30 °C with R2A medium. Strain 5420S-30^T grew on nutrient agar (NA; Difco) and R2A (Difco), but not on trypticase soy agar (TSA; Difco) or MacConkey agar (Difco). Gram staining, catalase and oxidase activities and hydrolysis of

casein, DNA, hypoxanthine, starch, Tween 80 and xanthine were examined according to the methods of Smibert & Krieg (1994). Hydrolysis of CM-cellulose (0.1 %; Sigma), chitin (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was also tested. Cell morphology and motility were studied by transmission electron microscopy (TEM) (model 912AB; LEO) and phase-contrast microscopy (AXIO; Zeiss), with cells grown for 2 days at 30 °C on R2A. The temperature range for growth was tested from 5 to 45 °C in increments of 5 °C. The pH range for growth was determined in the range pH 4–10 in increments of 1 pH unit. Tolerance of NaCl was tested at 0, 1, 2, 3 and 5 % NaCl (w/v). Tests in the commercial API 20NE, API ID 32GN, API 50 CH and API ZYM systems (bioMérieux) were generally performed according to the manufacturer's instructions. Sensitivity to antibiotics was determined with the routine disc-diffusion plate method. The following antibiotics were tested: amikacin (30 µg), ampicillin (10 µg), cefotaxime (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (15 µg), nalidixic acid (30 µg), neomycin (30 µg), penicillin (10 U), polymyxin B (300 U), rifampicin (5 µg), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5420S-30^T is EF661571.

Cells of strain 5420S-30^T were Gram-negative, strictly aerobic, non-motile cocci, 1.2–1.5 µm in diameter. Colonies were pink, round and convex with clear margins. Differential phenotypic characteristics of strain 5420S-30^T and other *Roseomonas* species are shown in Table 1.

For quantitative analysis of whole-cell fatty acids, strain 5420S-30^T was cultivated on R2A at 30 °C for 48 h. Cellular fatty acid profiles were determined by using the MIDI microbial identification system (Microbial ID). The procedure for identification of isoprenoid quinones followed an HPLC method described previously (Groth *et al.*, 1996). The DNA G + C content was measured by HPLC (Mesbah *et al.*, 1989). The main fatty acids of 5420S-30^T were C_{18:1}ω7c (33.4 %), summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH; 32.0 %) and C_{16:0} (12.3 %) (Table 2). Strain had ubiquinone 10 (Q-10) as the predominant isoprenoid quinone. The DNA G + C content was 72.1 mol%.

The nearly complete 16S rRNA gene sequence (1354 bp) of strain 5420S-30^T was determined as described by Kwon

et al. (2003). Phylogenetic analysis was carried out using MEGA 3 (Kumar *et al.*, 2004) after multiple alignments of data by CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic dendrograms were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with bootstrap values based on 1000 replications. Sequence analyses based on the 16S rRNA gene sequence showed that strain 5420S-30^T fell within the radiation of the genus *Roseomonas* (Fig. 1). It showed 91.6–95.1 % sequence similarity to the type strains of recognized *Roseomonas* species, with the highest sequence similarities (95.1 and 94.5 %, respectively) to *R. aquatica* TR53^T and *R. gilardii* ATCC 49956^T.

Characteristically, strain 5420S-30^T was shown to be intolerant to NaCl, to be poor in substrate assimilation and not to degrade urea (Table 1). The fatty acid compositions of members of the genus *Roseomonas* vary considerably between species. The fatty acid composition of strain 5420S-30^T was similar to that of *R. aquatica* JCM

Table 1. Differential phenotypic characteristics among strain 5420S-30^T (*Roseomonas aerilata* sp. nov.) and recognized species of the genus *Roseomonas*

Strains: 1, 5420S-30^T; 2, *R. aquatica* JCM 13556^T; 3, *R. cervicalis* CIP 104027^T; 4, *R. gilardii* CIP 104026^T; 5, *R. lacus* JCM 13283^T; 6, *R. mucosa* CCUG 48654^T; 7, *R. terrae* KCTC 12874^T (data from Yoon *et al.*, 2007). Antibiotic susceptibility results were obtained from Yoon *et al.* (2007); other data were determined in this study unless indicated. All strains are negative for indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase (API 20NE test strips). +, Positive; –, negative; w, weak reaction; ND, not determined/no data available; v, variable.

Characteristic	1	2	3	4	5	6	7
Isolation source	Air	Potable water*	Cervix*	Potable water*	Freshwater lake sediment*	Blood*	Soil
Motility	–	–*	+	v*	–*	+	–
Growth on NaCl (%)	<1	<2*	≤6*	≤6*	>6.5*	ND*	≤1
Nitrate reduction	–	–	–	–	+	–	–
Urease	–	+	+	+	+	+	+
Assimilation of:							
D-Glucose	–	–	–	+	–	–	–
L-Arabinose	–	+	+	+	–	+	–
Potassium gluconate	–	+	+	+	+	+	–
Adipic acid	–	+	+	–	+	–	–
Malic acid	–	+	+	+	+	+	–
Trisodium citrate	–	–	+	+	–	+	–
Phenylacetic acid	–	–	–	+	–	+	ND
Suberic acid	+	–	+	–	+	+	ND
3-Hydroxybenzoic acid	+	–	–	+	–	+	ND
Susceptibility to:							
Ampicillin	–	–	–	v	–	ND	–
Chloramphenicol	+	+	ND	ND	+	ND	+
Cephalothin	+	–	–	–	–	ND	–
Kanamycin	+	+	ND	ND	+	ND	+
Neomycin	w	w	ND	ND	+	ND	+
Polymyxin B	–	–	ND	ND	–	ND	–
Streptomycin	+	+	ND	ND	+	ND	+
Tetracycline	+	+	+	+	+	ND	+
DNA G + C content (mol%)	72.1	68.6*	70.4*	67.6*	71.9*	ND	69.3

*Data from Gallego *et al.* (2006).

Table 2. Fatty acid compositions of strain 5420S-30^T (*R. aerilata* sp. nov.) and recognized species of the genus *Roseomonas*

Strains: 1, 5420S-30^T; 2, *R. aquatica* JCM 13556^T; 3, *R. cervicalis* CIP 104027^T; 4, *R. gilardii* CIP 104026^T; 5, *R. lacus* JCM 13283^T; 6, *R. mucosa* CCUG 48654^T; 7, *R. terrae* KACC 12677^T. Cells were harvested after growth on R2A medium for 2 days. Only fatty acids that represent more than 1 % of total fatty acids are shown. Data were obtained in this study.

Fatty acid	1	2	3	4	5	6	7
C _{12:0}	1.3	1.0	—	—	—	—	—
C _{14:0}	—	1.0	—	—	—	—	—
C _{16:0}	12.3	16.7	7.9	20.2	5.4	16.9	8.5
C _{16:0} 2-OH	—	1.7	—	—	—	—	1.4
C _{16:0} 3-OH	—	1.5	2.3	2.1	1.3	3.2	1.7
C _{16:1} ω5c	—	—	3.3	—	4.3	—	2.2
C _{17:0} cyclo	3.1	—	—	—	—	—	—
C _{18:0}	5.6	4.9	—	—	—	—	—
C _{18:0} 3-OH	3.4	—	—	—	—	—	—
C _{18:1} 2-OH	6.7	7.7	15.0	4.0	13.8	9.0	13.4
C _{18:1} ω5c	—	1.0	—	—	—	—	—
C _{18:1} ω7c	33.4	37.8	64.3	43.7	67.4	63.4	63.3
C _{18:1} ω9c	—	1.3	—	—	—	—	—
C _{18:1} ω7c 11-methyl	—	—	—	—	3.5	—	—
C _{19:0} ω8c cyclo	—	—	—	25.6	—	5.2	6.7
C _{20:2} ω6,9c	—	—	—	—	—	—	—
Summed feature 2*	1.6	—	—	—	—	—	—
Summed feature 3*	32.0	20.0	3.8	1.2	4.2	1.4	1.0
Summed feature 4*	—	—	—	—	—	—	—
Summed feature 5*	—	2.6	—	—	—	—	—
Unknown 10.928	—	1.1	1.3	—	—	1.0	—

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contained C_{14:0} 3-OH and/or iso-C_{16:1} I. Summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 4 contained iso-C_{17:1} I and/or anteiso-C_{17:1} B. Summed feature 5 contained C_{18:2}ω6,9c and/or anteiso-C_{18:0}.

13556^T, with C_{18:1}ω7c, summed feature 3 and C_{16:0} predominant (Table 2).

On the basis of this polyphasic taxonomic approach, strain 5420S-30^T represents a novel species of the genus *Roseomonas*, for which the name *Roseomonas aerilata* sp. nov. is proposed.

Description of *Roseomonas aerilata* sp. nov.

Roseomonas aerilata (ae.ri.la'ta. L. n. *aer* air; L. part. fem. adj. *lata* carried; N.L. part. fem. adj. *aerilata* airborne).

Cells are Gram-negative, strictly aerobic, non-motile cocci, 1.2–1.5 µm in diameter. Colonies are pink, round and convex with clear margins. Growth occurs at 5–35 °C (optimum 30 °C) and pH 5–9 (optimum pH 6–7). Growth does not occur in as little as 1 % (w/v) NaCl. Catalase- and oxidase-positive. Decomposes Tween 80. Does not decompose casein, chitin, CM-cellulose, DNA, hypoxanthine, pectin, starch, tyrosine or xanthine. Shows negative reactions for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, hydrolysis of urea, aesculin and gelatin and β-galactosidase (API 20NE test strips). Assimilates only suberic acid, 3-hydroxybenzoic acid and 3-hydroxybutyric acid of the substrates in API 20NE and API ID 32GN test strips. Positive for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities in API ZYM test strips. Does not produce any acids in API 50 CH test strips. Resistant to ampicillin (10 µg), fusidic acid (10 µg), lincomycin (15 µg), polymyxin B (300 U) and vancomycin (30 µg). Weakly sensitive to neomycin (30 µg) and sensitive to amikacin (30 µg), cefotaxime (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), penicillin (10 U), rifampicin (5 µg), streptomycin (10 µg) and tetracycline (30 µg). The major fatty acids are C_{18:1}ω7c (33.4 %), summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH; 32.0 %) and C_{16:0} (12.3 %). The major isoprenoid quinone is Q-10. The DNA G + C content of the type strain is 72.1 mol%.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain 5420S-30^T and closely related species. Asterisks indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bootstrap values below 50 % are not indicated. Bar, 0.02 changes per nucleotide position.

The type strain is 5420S-30^T (=KACC 12521^T =DSM 19363^T), isolated from air in the Republic of Korea.

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