Rubellimicrobium aerolatum sp. nov., isolated from an air sample in Korea

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A pink-coloured bacterial strain, designated 5715S-9^T, was isolated from an air sample collected in Suwon, Republic of Korea. The cells were strictly aerobic, Gram-negative, non-spore-forming, non-flagellated and short-rod-shaped. On the basis of 16S rRNA gene sequence analysis, the organism was a member of the genus *Rubellimicrobium*, showing the highest sequence similarities with *Rubellimicrobium mesophilum* MSL-20^T (96.2% sequence similarity) and *Rubellimicrobium thermophilum* DSM 16684^T (93.9%). The major polar lipids were phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid. The major fatty acids (>10%) were C_{18:1}ω7c, C_{16:0} and 11-methyl C_{18:1}ω7c. The G + C content of the DNA was 69 mol%. On the basis of both phylogenetic and phenotypic evidence, strain 5715S-9^T represents a novel species of the genus *Rubellimicrobium*, for which the name *Rubellimicrobium aerolatum* sp. nov. is proposed. The type strain is 5715S-9^T (=KACC 12504^T=DSM 19297^T).

The *Roseobacter* clade or the ‘*Roseobacter*–*Sulfitobacter*–*Silicibacter*’ group (Wagner-Döbler *et al.*, 2003) was named after the genus *Roseobacter* by Giovannoni & Rappé (2000). The members of this group were found largely in marine environments (González & Moran, 1997; Eilers *et al.*, 2000; Rappé *et al.*, 2000; Zubkov *et al.*, 2001). Among them, two closely related novel members, *Rubellimicrobium thermophilum* (Denner *et al.*, 2006) and *Wenxinia marina* (Ying *et al.*, 2007), were reported recently, having been isolated from biofilms from fine-paper machines and a pulp dryer, and from an oilfield sediment from the South China Sea, respectively. Most recently, a novel member of the genus *Rubellimicrobium*, *Rubellimicrobium mesophilum*, was proposed for a soil isolate (Dastager *et al.*, 2008).

In our study of the microbial diversity of air samples collected in the Suwon region of the Republic of Korea, a pink-coloured strain, designated 5715S-9^T, was obtained. Air samples were collected with an MAS-100 air sampler (Merck) (single-stage multiple-hole impactor) containing Petri dishes with R2A agar (BBL) amended with 200 µg cycloheximide

ml⁻¹ (Sigma). After sampling, the plates were incubated at 30 °C for 5 days and strain 5715S-9^T was recovered.

The 16S rRNA gene was amplified by using PCR with primers fD1 and rP2 (Weisburg *et al.*, 1991) on colonies; the entire PCR fragment was directly sequenced (Hiraishi, 1992). The closest known relatives of the novel isolate were determined by performing GenBank/EMBL/DDBJ database searches. After multiple alignments of the data had been performed using CLUSTAL W (Thompson *et al.*, 1994), the software package MEGA, version 3.1 (Kumar *et al.*, 2004), was used for all analyses. Phylogenetic dendrograms were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods and bootstrap percentages based on 1000 replications. For strain 5715S-9^T, 1354 bp of the 16S rRNA gene sequence were determined. The strain was found to be a member of the genus *Rubellimicrobium*. The highest levels of sequence similarity for strain 5715S-9^T occurred with respect to *Rubellimicrobium mesophilum* MSL-20^T (96.2% sequence similarity), *Rubellimicrobium thermophilum* DSM 16684^T (93.9%) and *W. marina* JCM 14017^T (91.5%); less than 91.0% similarity was shown with respect to the type strains of all the other taxa tested. In the neighbour-joining tree (Fig. 1), strain 5715S-9^T clearly formed a lineage with *Rubellimicrobium mesophilum* and *Rubellimicrobium thermophilum*, as shown by the 100% bootstrap support. This result was also supported by the maximum-parsimony tree.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5715S-9^T is EU338486.

A transmission electron micrograph of cells of strain 5715S-9^T and two-dimensional thin-layer chromatograms of polar lipid extracts obtained from strain 5715S-9^T and *Rubellimicrobium thermophilum* DSM 16684^T are available as supplementary material with the online version of this paper.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain 5715S-9^T and some members of the *Alphaproteobacteria*. Bootstrap percentages (based on 1000 replications) >40% are shown at branch points. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.02 substitutions per nucleotide position.

Gram staining, catalase and oxidase activities and hydrolysis of carboxymethylcellulose, casein, chitin (from crab shells), DNA, hypoxanthine, pectin, starch, Tween 80, tyrosine and xanthine were tested using the methods of Smibert & Krieg (1994). Cell morphology and motility were investigated under a phase-contrast microscope and with transmission electron microscopy, using cells negatively stained with 0.5% uranyl acetate after growth on R2A medium. The optimum pH range for growth was examined in R2A broth adjusted to various pH values (pH 4–10, using increments of 1.0 pH unit). Strain 5715S-9^T was tested for its tolerance of NaCl by using R2A broth at various NaCl concentrations (0, 1, 2, 3 and 5%, w/v). The optimum temperature for growth of the strain was determined by testing various temperatures (5–45 °C, using increments of 5 °C) on R2A agar. Growth under anaerobic conditions was determined after incubating the strain in a GasPak (BBL) jar at 30 °C for 15 days. Physiological and biochemical properties were determined further using API ZYM, API 20NE, API 50 CH and API ID 32GN (all from bioMérieux). Tests in the commercial systems were generally performed according to the manufacturer's instructions. Whole-cell fatty acids were analysed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1997). Isoprenoid quinones were extracted and analysed as described by Groth *et al.* (1996). The DNA G + C content was determined by means of HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco).

Strain 5715S-9^T grew on R2A but not on nutrient agar (Difco), tryptic soy agar (Difco) or MacConkey agar (Difco). Transmission electron microscopy revealed that cells of strain 5715S-9^T had no flagella (see Supplementary Fig. S1, available in IJSEM Online). Differential physiological characteristics for strain 5715S-9^T and the species most closely related to it are given in Table 1. The major cellular fatty acids of strain 5715S-9^T were C_{18:1}ω7c (56.5%), C_{16:0} (16.6%) and 11-methyl C_{18:1}ω7c (10.4%). The detailed fatty acid compositions of strain 5715S-9^T and its close phylogenetic neighbours are shown in Table 2. The main isoprenoid quinone was Q-10. The polar lipids comprised phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid; two-dimensional thin-layer chromatograms of the polar lipids from strain 5715S-9^T and *Rubellimicrobium thermophilum* DSM 16684^T are shown in Supplementary Fig. S2 (available in IJSEM Online). The DNA G + C content was 69 mol%.

According to the phylogenetic tree (Fig. 1), strain 5715S-9^T belongs in the genus *Rubellimicrobium*. However, it could be differentiated from the closest relative, *Rubellimicrobium mesophilum*, on the basis of the presence of catalase and oxidase, growth at 35 °C and the differences in fatty acid compositions. In addition, the strain is clearly separable from *Rubellimicrobium thermophilum* on the basis of the colony colour (pink versus red), the absence of flagella, the dearth of hydrolytic and assimilative activities and the fatty acid compositions (Tables 1 and 2). On the basis of the

Table 1. Differential phenotypic characteristics of strain 5715S-9^T and closely related taxa

Strains: 1, 5715S-9^T (*Rubellimicrobium aerolatum* sp. nov.); 2, *Rubellimicrobium mesophilum* MSL-20^T (data from Dastager *et al.*, 2008); 3, *Rubellimicrobium thermophilum* DSM 16684^T (Denner *et al.*, 2006; this study); 4, *W. marina* HY34^T (Ying *et al.*, 2007). +, Positive; (+), weakly positive; –, negative; ND, not determined.

Characteristic	1	2	3	4
Source of isolation	Air	Soil	Biofilms*	Marine sediment
Colony colour	Pink	Pink to light reddish	Red*	Faint pink
Flagellation	–	–	Polar flagella*	–
Catalase/oxidase	+ / +	– / –	+ / +	+ / +
Growth at 35 °C	+	–	+	+
Tolerance of NaCl (%)	≤1	<1	ND	≤9
Nitrate reduction	–	–	–	+
Urease	–	ND	–	+
Hydrolysis of:				
Aesculin	–	ND	+	ND
Gelatin	–	ND	+	ND
Assimilation of:				
L-Alanine	–	ND	+	(+)
L-Arabinose	–	ND	+	ND
L-Fucose	–	ND	+	+
D-Glucose	–	ND	+	ND
Lactic acid	–	ND	+	+
Malic acid	–	–	–	+
D-Mannitol	–	ND	+	–
D-Mannose	–	–	+	ND
Melibiose	–	–	+	(+)
Potassium gluconate	–	ND	+	+
L-Rhamnose	–	–	+	+
Sucrose	–	ND	+	+
D-Sorbitol	–	–	+	(+)
DNA G + C content (mol%)	69	72.3	69.4–70.2	69.4

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

phenotypic and phylogenetic data presented in this study, strain 5715S-9^T represents a novel species within the genus *Rubellimicrobium*, for which the name *Rubellimicrobium aerolatum* sp. nov. is proposed.

Description of *Rubellimicrobium aerolatum* sp. nov.

Rubellimicrobium aerolatum (ae.ro.la'tum. Gr. n. *aer* air; L. part. adj. *latus* -a -um carried; N.L. part. adj. *aerolatum* airborne).

Cells are strictly aerobic, Gram-negative, non-motile, non-spore-forming, short rods (0.8 µm wide and 1.6–3.6 long). Colonies are pink-coloured and circular with clear margins. Oxidase- and catalase-positive. Temperature and pH ranges for growth are 5–35 °C and pH 6–7, with optima at 30 °C and pH 7. Growth occurs in the presence of 0–1 % NaCl, but not with 2 % NaCl. Weakly hydrolyses xanthine but not casein, chitin, carboxymethylcellulose, DNA, hypoxanthine, pectin, starch, tyrosine or Tween 80. Positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation,

arginine dihydrolase, urease, aesculin hydrolase and gelatin hydrolase (API 20NE). Does not assimilate any substrates in API 20NE and API ID 32GN kits. Does not form any acids from any of the substrates in the API 50 CH kit. Positive for β-glucosidase, weakly positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase and negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). The major isoprenoid quinone system is Q-10. Major polar lipids are phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid. Major fatty acids (>10 %) are C_{18:1ω7c}, C_{16:0} and 11-methyl C_{18:1ω7c}. The DNA G + C content of the type strain is 69 mol%.

The type strain, 5715S-9^T (=KACC 12504^T=DSM 19297^T), was isolated from an air sample collected in the Suwon region of the Republic of Korea.

Table 2. Cellular fatty acid contents of strain 5715S-9^T and closely related strains

Strains: 1, 5715S-9^T (*Rubellimicrobium aerolatum* sp. nov.); 2, *Rubellimicrobium mesophilum* MSL-20^T (data from Dastager *et al.*, 2008); 3, *Rubellimicrobium thermophilum* DSM 16684^T; 4, *W. marina* HY34^T (Ying *et al.*, 2007). Cells of strains 5715S-9^T and DSM 16684^T were harvested after cultivation on R2A at 30 °C for 4 days. Data are expressed as percentages of total fatty acids; fatty acids constituting more than 1 % of total fatty acids are shown. –, <1 % or not detected.

Fatty acid	1	2	3	4
C _{10:0}	–	1.3	–	–
C _{10:0} 3-OH	4.7	–	2.2	–
C _{12:0}	–	1.3	–	1.6
C _{13:0} 2-OH	–	–	–	1.2
C _{14:0}	–	–	–	3.7
iso-C _{15:1} G	–	–	–	3.4
C _{17:0}	–	1.2	–	–
C _{16:0}	16.6	36.9	7.8	16.5
C _{18:0}	2.3	3.6	14.6	3.9
C _{18:1} ω7c	56.5	36.5	45.6	57.1
11-Methyl C _{18:1} ω7c	10.4	12.4	–	5.4
C _{19:0} ω8c cyclo	–	–	22.3	–
Unknown fatty acids*				
ECL 10.928	4.1	–	–	–
ECL 11.799	–	–	2.8	–
Summed features†				
3	2.4	–	–	1.9
5	1.1	–	–	–

*ECL, Equivalent chain-length.

†Summed features are groups of two or three fatty acids that cannot be separated using the MIDI system: summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 5 comprises C_{18:2}ω6,9c and/or anteiso-C_{18:0}.

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