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Description of *Microvirga aerophila* sp. nov. and *Microvirga aerilata* sp. nov., isolated from air, reclassification of *Balneimonas flocculans* Takeda *et al.* 2004 as *Microvirga flocculans* comb. nov. and emended description of the genus *Microvirga*

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Two bacterial strains, 5420S-12^T and 5420S-16^T, isolated from air samples, were characterized using a polyphasic approach. 16S rRNA gene sequence analysis showed that strain 5420S-12^T was related phylogenetically to *Microvirga subterranea* Fail4^T (97.4 % sequence similarity) and *Microvirga guangxiensis* 25B^T (97.1 %) and that strain 5420S-16^T was closely related to *Balneimonas flocculans* TFB^T (98.0 %) and *Microvirga guangxiensis* 25B^T (97.2 %). The G+C content of the genomic DNA was 62.2 mol% for strain 5420S-12^T and 61.5 mol% for strain 5420S-16^T. The major fatty acid was $C_{18:1}\omega7c$. The results of DNA–DNA hybridization and the phenotypic data showed that strains 5420S-12^T and 5420S-16^T could be distinguished from phylogenetically related species and represent two novel species within the genus *Microvirga*, for which the names *Microvirga aerophila* sp. nov. (type strain 5420S-12^T =KACC 12743^T =NBRC 106136^T) and *Microvirga aerilata* sp. nov. (type strain 5420S-16^T =KACC 12744^T =NBRC 106137^T) are proposed. Furthermore, the reclassification of *Balneimonas flocculans* as *Microvirga flocculans* comb. nov. (type strain TFB^T =JCM 11936^T =KCTC 12101^T =IAM 15034^T =ATCC BAA-817^T) is proposed and an emended description of the genus *Microvirga* is provided.

The genus *Microvirga* was proposed by Kanso & Patel (2003), with the type species *Microvirga subterranea*, for an isolate from geothermal waters. Shortly afterwards, its closest neighbour, '*Balneomonas flocculans*', was isolated from a hot spring (Takeda *et al.*, 2004a). The genus name '*Balneomonas*' was corrected to *Balneimonas* upon valid publication according to Rule 61 of the Bacteriological Code and the name was listed in Validation List no. 97 (Takeda *et al.*, 2004b). The two genera were reported in different journals and, hence, their taxonomic properties were not compared despite their close phylogenetic relatedness (96.3 % 16S rRNA gene sequence similarity). Recently, another strain, 25B^T, isolated from a rice-field soil sample, was assigned to the novel species *Microvirga guangxiensis* (Zhang *et al.*, 2009). In this study, we report the taxonomic characterization of two airborne bacterial strains, 5420S-12^T and 5420S-16^T, and the taxonomic reassignment of *Balneimonas flocculans* as a member of the genus *Microvirga*.

During a course of study on the microbial diversity of the atmosphere in the Suwon region of the Republic of Korea, air samples were collected with an MAS-100 air sampler (Merck; single-stage multiple-hole impactor) containing Petri dishes with R2A agar (Difco) amended with 200 μg cycloheximide ml^{-1} (Sigma). After sampling, the plates were incubated at 28 °C for 5 days and pink-coloured strains 5420S-12^T and 5420S-16^T were recovered. Routine cultivation was conducted at 28 °C with R2A agar.

Phenotypic characteristics, including Gram-staining, catalase and oxidase activity and hydrolysis of CM-cellulose

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 5420S-12^T and 5420S-16^T are GQ421848 and GQ421849.

Results of 2D TLC of polar lipids of the novel strains and related type strains are available as supplementary material with the online version of this paper.

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(0.1 %, w/v), casein (10 %, w/v, skimmed milk), chitin (0.5 %, w/v), hypoxanthine (0.5 %, w/v), pectin (0.5 %, w/v), tyrosine (0.5 %, w/v), Tween 80 (1.0 %, v/v), starch (1.0 %, w/v) and xanthine (0.5 %, w/v), were determined using the methods of Smibert & Krieg (1994). DNase activity was determined with DNase test agar (Difco). Growth under anaerobic conditions was tested in a GasPak jar (BBL) at 28 °C for 14 days. The pH range (pH 4.0–10.0 at intervals of 1.0 pH unit) for growth was determined in R2A broth buffered with citrate/phosphate buffer or Tris/HCl (Breznak & Costilow, 1994). Growth at 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 % NaCl (w/v) and at 5–50 °C (at intervals of 5 °C) was investigated on R2A agar. Tests in the commercial systems API 20NE, API ID 32GN and API ZYM (bioMérieux) were performed according to the manufacturer's instructions. The API ZYM test strip was read after 4 h of incubation at 37 °C, whilst the other API strips were examined after 7 days at 28 °C. Cell morphology was observed by transmission electron microscopy (912AB; LEO) and phase-contrast microscopy (AXIO; Zeiss) with cells grown on R2A agar. Motility was tested by the hanging-drop method (Skerman, 1967) using the phase-contrast microscope.

Strains 5420S-12^T and 5420S-16^T grew on R2A agar and nutrient agar (NA), but not on trypticase soy agar or MacConkey agar (all from Difco). In the API 20NE and ID 32GN strips, strains 5420S-12^T and 5420S-16^T did not assimilate any of the available substrates (up to 14 days of incubation). Detailed characteristics for the two strains are given in the species descriptions.

Fatty acid methyl esters were extracted and prepared by using the standard protocol of the Microbial Identification system (MIDI, 1999) after cells were grown on R2A agar (Difco) for 3 days at 28 °C. Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). Polar lipids were analysed according to Minnikin *et al.* (1984). The DNA G+C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18 S; Supelco).

Strains 5420S-12^T and 5420S-16^T contained $C_{18:1}\omega7c$ and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) as the major fatty acids (Table 1). Q-10 was the major ubiquinone for both strains. The polar lipid patterns of the two strains were similar, with phosphatidylcholine and phosphatidylethanolamine as the major components (see Supplementary Fig. S1, available in IJSEM Online). Additionally, phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine, diphosphatidylglycerol and phosphatidylglycerol were detected in moderate amounts. These profiles were consistent with those of *M. subterranea* DSM 14364^T and *B. flocculans* KCTC 12101^T (Supplementary Fig. S1). The DNA G+C contents of strains 5420S-12^T and 5420S-16^T were 62.2 and 61.5 mol%, respectively, which were a little lower than the values reported for the genus *Microvirga* (63.5–64.3 mol%).

16S rRNA gene sequences were determined by PCR amplification (Kwon *et al.*, 2003) and direct sequencing (Hiraishi, 1992). Nearly complete 16S rRNA gene sequences were determined for strains 5420S-12^T and 5420S-16^T (both 1439 bp). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). CLUSTAL W version 1.8 (Thompson *et al.*, 1994) was used to align the sequences of strains 5420S-12^T and 5420S-16^T with those of related taxa retrieved from public databases. Phylogenetic analysis was performed using the software package MEGA version 3.1 (Kumar *et al.*, 2004) using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with bootstrap values (Felsenstein, 1985) based on 1000 replications. To determine genomic relatedness, the filter hybridization method was performed according to Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using the Bio-1D Image analysis software (Vilber Lourmat).

A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences revealed that strains 5420S-12^T and 5420S-16^T were members of the family *Methylobacteriaceae* of the *Alphaproteobacteria*. The two strains formed a robust cluster (bootstrap value 100 %) with *M. subterranea* FaiI4^T, *M. guangxiensis* 25B^T and *B. flocculans* TFB^T (Fig. 1). Similar results were obtained with the maximum-parsimony algorithm. The 16S rRNA gene sequence similarity between strains 5420S-12^T and 5420S-16^T was 96.6 %. Strain 5420S-12^T exhibited 97.4, 97.1 and 95.9 % similarity to *M. subterranea* FaiI4^T, *M. guangxiensis* 25B^T and *B. flocculans* TFB^T, respectively, while strain 5420S-16^T showed 98.0, 97.2 and 96.8 % similarity to *B. flocculans* TFB^T, *M. guangxiensis* 25B^T and *M. subterranea* FaiI4^T, respectively.

To clarify the taxonomic position at the species level, DNA–DNA relatedness was examined. Strain 5420S-12^T showed 48.0 % (reciprocal 50.8 %) DNA–DNA relatedness to *M. subterranea* DSM 14364^T and 40.9 % to *M. guangxiensis* JCM 15710^T (reciprocal 30.2 %). Strain 5420S-16^T showed low levels of DNA–DNA relatedness, 39.0 % (reciprocal 36.8 %) and 35.6 % (reciprocal 13.3 %), respectively, to *B. flocculans* KCTC 12101^T and *M. guangxiensis* JCM 15710^T. The 16S rRNA gene-based phylogeny and DNA–DNA hybridization results indicated that strains 5420S-12^T and 5420S-16^T could be classified as novel members of the genera *Microvirga* or *Balneimonas*.

B. flocculans TFB^T showed 96.3 and 96.0 % 16S rRNA gene sequence similarity to *M. subterranea* FaiI4^T and *M. guangxiensis* 25B^T, respectively. *B. flocculans* KCTC 12101^T shared similar physiological characteristics and fatty acid and polar lipid patterns and the same quinone system (Q-10) with the genus *Microvirga*. On the basis of the phenotypic

Table 1. Cellular fatty acid contents of strains 5420S-12^T and 5420S-16^T and type strains of related species

Strains: 1, *Microvirga aerophila* sp. nov. 5420S-12^T; 2, *Microvirga aerilata* sp. nov. 5420S-16^T; 3, *M. subterranea* DSM 14364^T; 4, *B. flocculans* KCTC 12101^T; 5, *M. guangxiensis* JCM 15710^T. Data were obtained from this study. Prior to fatty acid extraction, all strains were grown on R2A agar (Difco) at 28 °C for 3 days. Values are percentages of total fatty acids. −, Not detected.

Fatty acid	1	2	3	4	5
$C_{14:0}$	1.1	0.9	−	−	0.8
$C_{15:1}\omega 8c$	−	0.6	−	−	0.3
$C_{16:0}$	7.6	9.8	5.5	7.3	9.9
$C_{17:0}$	−	−	6.7	1.1	0.6
$C_{17:0}$ cyclo	1.1	−	−	−	0.3
$C_{17:1}\omega 6c$	−	−	1.4	−	−
$C_{17:1}\omega 8c$	−	−	2.0	−	−
$C_{18:0}$	1.0	2.1	3.8	4.9	6.0
$C_{18:0}$ 3-OH	0.8	−	−	2.3	1.1
$C_{18:1}\omega 7c$	69.6	71.8	73.4	63.3	63.4
$C_{19:0}$ cyclo $\omega 8c$	3.6	−	2.4	6.6	11.1
$C_{19:0}$ 10-methyl	−	0.9	−	1.5	0.3
$C_{20:2}\omega 6,9c$	−	−	−	−	−
Summed features*					
Summed feature 2	4.3	3.7	3.3	9.5	1.9
Summed feature 3	11.0	10.4	1.5	1.7	2.3
Summed feature 7	−	−	−	1.9	−
Unknown†					
ECL 10.928	−	−	−	−	0.5
ECL 14.502	−	−	−	−	0.2
ECL 14.959	−	−	−	−	1.3

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI system. Summed feature 2 comprises $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; summed feature 3 comprises $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 7 comprises unknown 18.846 and/or $C_{19:1}\omega 6c$.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified; equivalent chain-lengths are given.

and phylogenetic data presented here, the genera *Microvirga* and *Balneimonas* could not be separated clearly. Hence, we propose that members of the genus *Balneimonas* should be reclassified as members of the genus *Microvirga* because the genus *Microvirga* (Kanso & Patel, 2003) has nomenclatural priority over the genus *Balneimonas* (Takeda *et al.*, 2004a, b) according to Principle 6 and Rule 24b(2) of the Bacteriological Code.

Strains 5420S-12^T and 5420S-16^T shared many physiological and morphological characteristics. However, the two strains could be differentiated from each other by NaCl ranges for growth and enzyme profiles (API ZYM) (Table 2). Strains 5420S-12^T and 5420S-16^T could be differentiated from other phylogenetically related species based on the hydrolysis of starch, temperature range for growth and enzyme activities (API ZYM). Also, the two strains could be clearly differentiated from other *Microvirga* species by means of their smaller amounts of $C_{18:0}$ and larger amounts of summed feature 3 ($C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH) (Table 1). On the basis of the data obtained in this study, strains 5420S-12^T and 5420S-16^T represent novel members of the genus *Microvirga*, for which the names *Microvirga aerophila* sp. nov. and *Microvirga aerilata* sp. nov. are proposed.

Emended description of the genus *Microvirga* Kanso and Patel 2003 emend. Zhang *et al.* 2009

The description remains as given by Kanso & Patel (2003) and Zhang *et al.* (2009) with the following modifications. The temperature range for growth is 10–45 °C. Positive for catalase, but negative for hydrolysis of casein, chitin, CM-cellulose and xanthine, indole production, glucose fermentation and arginine dihydrolase. The G+C content of the DNA is 61.5–64.3 mol%. The predominant isoprenoid quinone is Q-10. The major fatty acid is $C_{18:1}\omega 7c$. The polar lipids consist of phosphatidylcholine and phosphatidylethanolamine as major components and phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine, diphosphatidylglycerol and phosphatidylglycerol in moderate amounts. The type species is *Microvirga subterranea.*

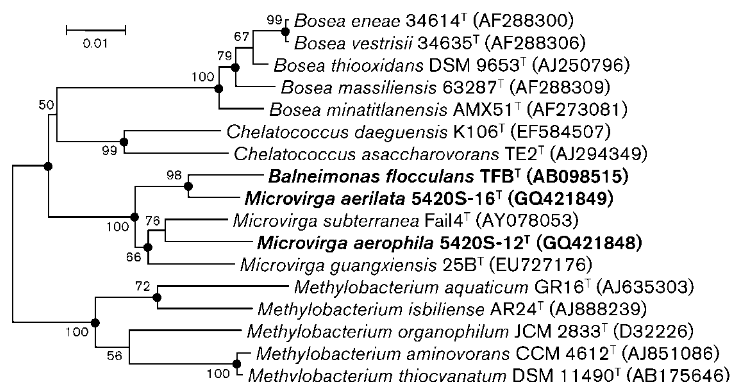

Fig. 1. Phylogenetic dendrogram constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships between strains 5420S-12^T and 5420S-16^T and related species. Bootstrap values (expressed as percentages of 1000 replications) ⩾50 % are shown at branch points. Dots indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 1 substitution per 100 nucleotide positions.

Table 2. Differential phenotypic characteristics of strains 5420S-12^T and 5420S-16^T and the type strains of closely related species

Strains: 1, *M. aerophila* sp. nov. 5420S-12^T; 2, *M. aerilata* sp. nov. 5420S-16^T; 3, *M. subterranea* DSM 14364^T (unless indicated, data from Kanso & Patel, 2003); 4, *B. flocculans* KCTC 12101^T (Takeda *et al.*, 2004); 5, *M. guangxiensis* JCM 15710^T (Zhang *et al.*, 2009). All strains are rod-shaped, strictly aerobic and positive for catalase, but negative for hydrolysis of casein, chitin, CM-cellulose and xanthine, indole production, glucose fermentation and arginine dihydrolase. +, Positive; W, weakly positive; −, negative.

Characteristic	1	2	3	4	5
Isolation source	Air	Air	Thermal aquifer	Hot spring	Soil
Growth media*	R2A, NA	R2A, NA	R2A§, Rouf's agar	R2A§, Mn^{2+}-free PYG, PY	LB, Rouf's agar, GYM agar
Colony colour†	LP	LP	LP	WH	LP
Motility	−	−	+	+	−
Ranges for growth					
Temperature (°C)	10–35	10–35	25–45	20–45‡	16–42
NaCl concentration (%)	0–2	0–3	0–1	0–1.5‡	0–2
pH	7–10	7–10	6–9‡	6–9	5.0–9.5
Hydrolysis of:					
Aesculin	−	−	−	+	−
Gelatin	−	W	+	+	−
Hypoxanthine	−	−	−‡	+‡	−‡
Starch	+	+	−	−‡	+‡
Tyrosine	−	−	W‡	−‡	−‡
Urea	−	−	−	−	+
Oxidase	+	+	−	+	+
Nitrate reduction	−	−	+	−	+
Enzyme activities (API ZYM)					
Alkaline phosphatase	−	+	−‡	+‡	−‡
Esterase lipase (C8)	−	+	+‡	+‡	+‡
Leucine arylamidase	−	+	+‡	+‡	+‡
Trypsin	−	+	−‡	+‡	−‡
Naphthol-AS-BI-phosphohydrolase	+	+	−‡	+‡	−‡
DNA G+C content (mol%)	62.2	61.5	63.5±0.5	64	64.3

*GYM, Glucose-yeast extract-malt extract; LB, Luria–Bertani medium; PY, peptone-yeast extract medium; PYG, peptone-yeast extract-glucose medium.
†LP, Light pink; WH, white.
‡Data from this study.
§Determined in this study. The composition of the medium was given in the original paper.

Description of *Microvirga flocculans* comb. nov.

Basonym: *Balneimonas flocculans* Takeda *et al.* 2004.

The description is based on that given for *Balneimonas flocculans* by Takeda *et al.* (2004a) and the emended description of the genus given above, with the following additions. Growth occurs at 20–45 °C and with 0–1.5 % NaCl. Hydrolyses hypoxanthine. Does not hydrolyse starch or xanthine.

The type strain is TFB^T (=JCM 11936^T =KCTC 12101^T =IAM 15034^T =ATCC BAA-817^T).

Description of *Microvirga aerophila* sp. nov.

Microvirga aerophila (ae.ro.phi′la. Gr. n. *aer* air; Gr. adj. *philos* loving; N.L. fem. adj. *aerophila* air-loving).

Displays the following properties in addition to those given in the emended genus description. Cells are strictly aerobic, Gram-stain-negative, non-motile, non-spore-forming rods, 0.8–1.1 μm in diameter and 1.6–4.2 μm long. Colonies are smooth, circular, convex and pink after 3 days at 30 °C on R2A agar. Grows on R2A agar and NA, but not on marine agar, trypticase soy agar or MacConkey agar. Grows at 10–35 °C, at pH 7.0–10.0 and with 0–2.0 % NaCl. Hydrolyses starch but not DNA, hypoxanthine, pectin, tyrosine or Tween 80. Positive (in API 20NE and API ZYM strips) for esterase (C4), acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative for alkaline phosphatase, esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. The DNA G+C content of the type strain is 62.2 mol%.

The type strain, 5420S-12^T (=KACC 12743^T =NBRC 106136^T), was isolated from an air sample from Suwon, Republic of Korea.

Description of *Microvirga aerilata* sp. nov.

Microvirga aerilata (ae.ri.la′ta. L. n. *aer* air; L. part. adj. *latus -a -um* carried; N.L. fem. part. adj. *aerilata* airborne).

Displays the following properties in addition to those given in the emended genus description. Cells are strictly aerobic, Gram-stain-negative, non-motile, non-spore-forming rods, 1.2–1.5 μm in diameter and 1.6–3.3 μm long. Colonies are smooth, circular, convex and pink after 3 days at 30 °C on R2A agar. Grows on R2A agar and NA, but not on marine agar, trypticase soy agar or MacConkey agar. Grows at 10–35 °C, at pH 7.0–10.0 and with 0–3.0 % NaCl. Hydrolyses starch. Does not hydrolyse DNA, hypoxanthine, pectin, tyrosine or Tween 80. Positive (in API 20NE and API ZYM strips) for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, and negative for lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. The DNA G+C content of the type strain is 61.5 mol%.

The type strain, 5420S-16^T (=KACC 12744^T =NBRC 106137^T), was isolated from an air sample from Suwon, Republic of Korea.

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