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 C18 : 1ω7c. 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* 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
(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 morpho-
logy 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-12T and 5420S-16T 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-12T and 5420S-16T 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-12T and 5420S-16T contained C18:1ω7c and
summed feature 3 (comprising C16:1ω7c and/or iso-C15:0
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 phosphatidylethanolamine and
phosphatidylethanolamine as the major components
(see Supplementary Fig. S1, available in IJSEM Online).
Additionally, phosphatidylmonomethylethanolamine, phos-
phatidyldimethylethanolamine, diphasatidylglycerol and
phosphatidylglycerol were detected in moderate amounts.
These profiles were consistent with those of M. subterranea
DSM 14364T and B. flocculans KCTC 12101T (Supple-
mentary Fig. S1). The DNA G+C contents of strains 5420S-12T
and 5420S-16T 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 sequenc-
ing (Hiraishi, 1992). Nearly complete 16S rRNA gene
sequences were determined for strains 5420S-12T and
5420S-16T (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-12T and 5420S-16T 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 related-
ness, 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 relat-
edness 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-12T and 5420S-
16T were members of the family Methylobacteriaceae of the
Alphaproteobacteria. The two strains formed a robust cluster
(bootstrap value 100 %) with M. subterranea Fai4T, M. guangxiensis 25BT and B. flocculans TFBT
(Fig. 1). Similar results were obtained with the max-
imum-parsimony algorithm. The 16S rRNA gene sequence
similarity between strains 5420S-12T and 5420S-16T was
96.6 %. Strain 5420S-12T exhibited 97.4, 97.1 and 95.9 %
similarity to M. subterranea Fai4T, M. guangxiensis 25BT
and B. flocculans TFBT, respectively, while strain 5420S-16T
showed 98.0, 97.2 and 96.8 % similarity to B. flocculans
TFBT, M. guangxiensis 25BT and M. subterranea Fai4T,
respectively.

To clarify the taxonomic position at the species level,
DNA–DNA relatedness was examined. Strain 5420S-12T
showed 48.0 % (reciprocal 50.8 %) DNA–DNA relatedness
to M. subterranea DSM 14364T and 40.9 % to M. guangxiensis
JCM 15710T (reciprocal 30.2 %). Strain 5420S-16T showed low levels of DNA–DNA relatedness,
39.0 % (reciprocal 36.8 %) and 35.6 % (reciprocal 13.3 %)
respectively, to B. flocculans KCTC 12101T and M.
guangxiensis JCM 15710T. The 16S rRNA gene-based
phylogeny and DNA–DNA hybridization results indicated
that strains 5420S-12T and 5420S-16T could be classified as
novel members of the genera Microvirga or Balneimonas.
B. flocculans TFBT showed 96.3 and 96.0 % 16S rRNA gene
sequence similarity to M. subterranea Fai4T and M.
guangxiensis 25BT, respectively. B. flocculans KCTC 12101T
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-12T and 5420S-16T and type strains of related species

Strains: 1. Microvirga aerophila sp. nov. 5420S-12T; 2. Microvirga aerilata sp. nov. 5420S-16T; 3. M. subterranea DSM 14364T; 4. B. flocculans KCTC 12101T; 5. M. guangxiensis JCM 15710T. 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.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.1</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
<td>0.8</td>
</tr>
<tr>
<td>C15:03-OH</td>
<td>–</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
<td>0.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>7.6</td>
<td>9.8</td>
<td>5.5</td>
<td>7.3</td>
<td>9.9</td>
</tr>
<tr>
<td>C17:0</td>
<td>–</td>
<td>–</td>
<td>6.7</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>1.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.3</td>
</tr>
<tr>
<td>C17:1 o8c</td>
<td>–</td>
<td>–</td>
<td>1.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:0</td>
<td>–</td>
<td>–</td>
<td>2.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>1.0</td>
<td>2.1</td>
<td>3.8</td>
<td>4.9</td>
<td>6.0</td>
</tr>
<tr>
<td>C18:0 5-OH</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
<td>2.3</td>
<td>1.1</td>
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<tr>
<td>C18:1 o7c</td>
<td>69.6</td>
<td>71.8</td>
<td>73.4</td>
<td>63.3</td>
<td>63.4</td>
</tr>
<tr>
<td>C19:0 cyclo</td>
<td>3.6</td>
<td>–</td>
<td>2.4</td>
<td>6.6</td>
<td>11.1</td>
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<tr>
<td>C19:0 10-methyl</td>
<td>–</td>
<td>0.9</td>
<td>–</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>C20:0 o6,9c</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed features*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 2</td>
<td>4.3</td>
<td>3.7</td>
<td>3.3</td>
<td>9.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>11.0</td>
<td>10.4</td>
<td>1.5</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Summed feature 7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.9</td>
<td>–</td>
</tr>
<tr>
<td>Unknown†</td>
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<td></td>
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<td></td>
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<tr>
<td>ECL 10.928</td>
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<td>–</td>
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<td>ECL 14.502</td>
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<td>–</td>
<td>0.2</td>
</tr>
<tr>
<td>ECL 14.959</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI system. Summed feature 2 comprises C14:0 3-OH and/or iso-C16:1; summed feature 3 comprises C16:0 3-OH and/or iso-C16:0 2-OH; summed feature 7 comprises unknown C15:0 3-OH and/or iso-C16:1 2-OH.
†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-12T and 5420S-16T 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-12T and 5420S-16T 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 C18:0 and larger amounts of summed feature 3 (C16:1 o7c and/or iso-C15:0 2-OH) (Table 1). On the basis of the data obtained in this study, strains 5420S-12T and 5420S-16T represent novel members of the genus Microvirga, for which the names Microvirga aerophila sp. nov. and Microvirga aerilata sp. nov. are proposed.


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 C18:1 o7c. The polar lipids consist of phosphatidylcholine and phosphatidyethanolamine as major components and phosphatidylmonomethylethanolamine, phosphatidylmethylethanolamine, diphasatidylglycerol and phosphatidylglycerol in moderate amounts. The type species is Microvirga subterranea.
**Table 2.** Differential phenotypic characteristics of strains 5420S-12<sup>T</sup> and 5420S-16<sup>T</sup> and the type strains of closely related species

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

*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.**


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<sup>T</sup> (=KCTC 12101<sup>T</sup> =IAM 15034<sup>T</sup> =ATCC BAA-817<sup>T</sup>).

**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<sup>T</sup> (=KACC 12743<sup>T</sup> =NBRC 106136<sup>T</sup>), was isolated from an air sample from Suwon, Republic of Korea.

http://ijs.sgmjournals.org
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, trypsicase 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-Bl-phosphohydrolase, and negative for lipase (C14), valine arylamidase, cystine arylamidase, z-chymotrypsin, z-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, z-mannosidase and z-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 106137T), was isolated from an air sample from Suwon, Republic of Korea.

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References


