**Rhodocytophaga aerolata gen. nov., sp. nov., a new member of the family Cytophagaceae isolated from air**

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A strictly aerobic, Gram-staining-negative, oxidase- and catalase-positive, non-motile, rod-shaped bacterium, designated strain 5416T-29ᵀ, was isolated from air and was characterized by using a polyphasic approach. Colonies were reddish pink and circular with entire margins. Flexirubin-type pigments were absent. The strain formed a distinct phylogenetic lineage within the family Cytophagaceae of the phylum Bacteroidetes. Strain 5416T-29ᵀ did not show more than 88 % 16S rRNA gene sequence similarity to the type strain of any recognized species. The major cellular fatty acids were C₁₆:₁(ω₅c), iso-C₁₇:₀3-OH and iso-C₁₅:₀. The polar lipids were phosphatidylethanolamine, one unknown amino lipid and several unknown polar lipids. Menaquinone-7 (MK-7) was the major respiratory quinone. The G+C content of the DNA of strain 5416T-29ᵀ was 45.5 mol%. Results of phenotypic and phylogenetic analyses clearly indicate that strain 5416T-29ᵀ represents a novel species of a new genus in the family Cytophagaceae, for which the name Rhodocytophaga aerolata gen. nov., sp. nov. is proposed. The type strain of Rhodocytophaga aerolata is 5416T-29ᵀ (=KACC 12507ᵀ =DSM 22190ᵀ).

Members of the phylum Bacteroidetes [previously known as the Cytophaga–Flavobacterium–Bacteroides (CFB) group] are widely distributed in nature, particularly in aquatic ecosystems (Glöckner et al., 1999; Kirchman, 2002; Yoon et al., 2005), where they play important roles in biogeochemical cycles and the degradation of complex biopolymers such as cellulose, chitin (Cottrell & Kirchman, 2000) and agar (Nedashkovskaya et al., 2003). Over the past few years, many members of the phylum have been reclassified (Bernardet et al., 1996; Bowman, 2000; Kämpfer et al., 2006). Ludwig et al. (2008) include four classes within the phylum Bacteroidetes, namely ‘Bacteroidia’, ‘Flavobacteria’, ‘Sphingobacteria’ and ‘Cytophagia’. The last of these includes three families, of which Cytophagaceae (previously ‘Flexibacteraceae’) is the largest with Cytophaga as the type genus.

During the course of a study on cultivable bacteria from air samples, one reddish-pink bacterium, designated strain 5416T-29ᵀ, was isolated and subjected to a polyphasic taxonomic investigation. The results from the present phenotypic and phylogenetic analyses indicate that strain 5416T-29ᵀ represents a novel species of a new genus in the family Cytophagaceae.

An air sample was taken outside in a highly built-up area of Suwon city, Republic of Korea. The air sample was collected with an MAS-100 air sampler (Merck; single-stage, multiple-hole impactor) equipped with a Petri dish containing R2A agar (BBL) supplemented with cycloheximide (200 µg ml⁻¹; Sigma). The Petri dish was incubated at 30 °C for 5 days, and a reddish-pink colony was selected and purified on R2A agar by using the streak plate method. Genomic DNA of the novel strain was isolated according to the method of Ausubel et al. (1987), except that lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA gene was amplified by using the universal primers fD1 and rP2 (Weisburg et al., 1991), and was sequenced as described by Kwon et al. (2003). Alignment of the 16S rRNA gene sequence of strain 5416T-29ᵀ was performed with the CLUSTAL W program (Thompson et al., 1994) following identification of its
phylogenetic neighbours by using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Evolutionary distances were calculated according to Kimura’s two-parameter model (Kimura, 1983). Phylogenetic trees were constructed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods in the program MEGA3 (Kumar et al., 2004), with bootstrap values determined based on 1000 replications (Felsenstein, 1985). An almost-complete 16S rRNA gene sequence of strain 5416T-29T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential characteristics between strain 5416T-29T and its closest phylogenetic relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of isolation</td>
<td>Air sample</td>
<td>Soil</td>
<td>Soil</td>
<td>Soil</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Reddish pink</td>
<td>Light yellow*</td>
<td>Dark orange</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>( + )*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flexirubin-type pigments</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gliding motility</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Urea</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>x-Glucosidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetylgalactosaminidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>45.5</td>
<td>36</td>
<td>42</td>
<td>39</td>
</tr>
</tbody>
</table>

*Data from the present study.

Fig. 1. Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing the phylogenetic position of strain 5416T-29T among members of the phylum Bacteroidetes. Numbers at nodes indicate percentages of 1000 bootstrap resamplings; only values >70% are shown. Bacteroides fragilis ATCC 25285T and Pedobacter heparinus DSM 23661 were used as an outgroup.
29T was obtained (1416 bp). Preliminary comparisons with 16S rRNA gene sequences deposited in the GenBank database indicated that strain 5416T-29T belonged to the family Cytophagaceae. 16S rRNA gene sequence analysis revealed that strain 5416T-29T was related most closely to Fulvivirga kasyanovii KMM 6220T (87.7% similarity) and Sporocytophaga myxococcoides DSM 11118T (87.4%) but exhibited less than 87% sequence similarity to other members of the phylum Bacteroidetes. A 16S rRNA gene sequence similarity of 95% or less is generally regarded as a cut-off value for allocating a strain to a new genus (Ludwig et al., 1998). Hence, strain 5416T-29T should be treated as a representative of a new genus. The overall topologies of the phylogenetic trees were similar irrespective of the algorithms used for their construction (data not shown).

Although strain 5416T-29T showed highest 16S rRNA gene sequence similarity to F. kasyanovii KMM 6220T, in the neighbour-joining phylogenetic tree it formed a monophyletic clade in the cluster containing S. myxococcoides DSM 11118T, Cytophaga aurantiaca DSM 3654T and Cytophaga hutchinsonii DSM 1761T (Fig. 1). Hence, these latter three strains were selected for comparative analysis and were used as reference strains for several biochemical tests and for fatty acid and polar lipid analyses.

Cell morphology was examined by using phase-contrast (AXIO; Zeiss) and transmission electron (LEO model 912AB) microscopy with cells grown for 3 days on R2A agar at 30°C. For transmission electron microscopy, cells were negatively stained with 0.5% (w/v) uranyl acetate. Gram staining, catalase and oxidase activities and hydrolysis of CM-cellulose, casein, chitin from crab shells, hypoxanthine, pectin, starch, Tween 80 and xanthine were assessed by using the methods described by Smibert & Krieg (1994). Degradation of DNA was investigated on DNase test agar (Difco) supplemented with 0.01% K2CO3 and were used as reference strains for several biochemical tests and for fatty acid and polar lipid analyses.

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Table 2. Fatty acid compositions (%) of strain 5416T-29T and its closest phylogenetic relatives

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>tr</td>
<td>tr</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>tr</td>
<td>tr</td>
<td>1.2</td>
<td>3.2</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>16.5</td>
<td>27.3</td>
<td>28.3</td>
<td>29.8</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>4.7</td>
<td>7.1</td>
<td>4.5</td>
<td>4.8</td>
</tr>
<tr>
<td>C16:0</td>
<td>3.8</td>
<td>5.8</td>
<td>7.3</td>
<td>4.6</td>
</tr>
<tr>
<td>C16:0 2-OH</td>
<td>tr</td>
<td>1.3</td>
<td>tr</td>
<td>1.1</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>34.7</td>
<td>15.5</td>
<td>34.5</td>
<td>34.9</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>tr</td>
<td>–</td>
<td>tr</td>
<td>2.2</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>10.0</td>
<td>10.0</td>
<td>5.4</td>
<td>4.1</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>17.3</td>
<td>21.3</td>
<td>7.4</td>
<td>8.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>–</td>
<td>2.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 4*</td>
<td>7.6</td>
<td>tr</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Unknown 13.565†</td>
<td>–</td>
<td>1.6</td>
<td>1.9</td>
<td>tr</td>
</tr>
<tr>
<td>Unknown 14.959†</td>
<td>–</td>
<td>1.6</td>
<td>tr</td>
<td>–</td>
</tr>
<tr>
<td>Unknown 16.582†</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
<td>tr</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 4 comprises iso-C17:1 I and/or anteiso-C17:1 B.
†Unknown fatty acids are designated by their equivalent chain-length (ECL).

Cellular fatty acid methyl esters were prepared from cells of strain 5416T-29T and the three reference strains after growth on R2A agar for 4 days at 30°C and were analysed by GC according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were analysed by HPLC as described by Groth et al. (1996). The polar lipid profiles of strain 5416T-29T, S. myxococcoides DSM 11118T and C. hutchinsonii DSM 1761T were determined according to the method of Minnikin et al. (1984). The DNA G+C content of strain 5416T-29T was determined by HPLC of deoxyribonucleosides as described by Mesbah et al. (1989), by using a reversed-phase column (Supelcosil LC-18 S; Supelco). Strain 5416T-29T could be differentiated from S. myxococcoides DSM 11118T, C. aurantiaca DSM 3654T and C. hutchinsonii DSM 1761T on the basis of relatively high levels of summed feature 4 (comprising iso-C17:1 I and/or anteiso-C17:1 B) and small amount of iso-C15:0 (Table 2). The polar lipid profile of strain 5416T-29T consisted of phosphatidylethanolamine, one unknown amino lipid and several unknown polar lipids.
lipids (Fig. 2). It could be differentiated from the profile of *S. myxococcoides* DSM 11118T based on a low level of the unknown polar lipid L6 and the absence of the unknown polar lipids L8 and L9 and from the profile of *C. hutchinsonii* DSM 1761T based on the presence of the unknown amino lipid A and lack of the unknown polar lipid L10. The major respiratory quinone of strain 5416T-29T was menaquinone-7 (MK-7), consistent with all members of the family Cytophagaceae. The DNA G+C content of strain 5416T-29T was 45.5 mol%, a value significantly higher than that of the *Sporocytophaga* and *Cytophaga* reference strains. On the basis of the phenotypic and phylogenetic data presented, strain 5416T-29T cannot be assigned to any recognized taxa, and we therefore suggest that it represents a novel species of a new genus within the family Cytophagaceae, for which the name *Rhodocytophaga aerolata* gen. nov., sp. nov. is proposed.

**Description of *Rhodocytophaga* gen. nov.**

*Rhodocytophaga* (Rh’o’do.cy.to.pha’ga. Gr. adj. rhodos rose-red; N.L. fem. n. *Cytophaga* a bacterial genus; N.L. fem. n. *Rhodocytophaga* rose-red *Cytophaga*, referring to the reddish pink colour of colonies and to the phylogenetic relationship with the genus *Cytophaga*).

Cells are strictly aerobic, Gram-staining-negative rods that lack flagellar and gliding motility. Oxidase- and catalase-positive. Flexirubin-type pigments are absent. The major cellular fatty acids are C16:1ω5c, iso-C17:0 3-OH and iso-C15:0. The polar lipids are phosphatidylethanolamine, one unknown amino lipid and several unknown polar lipids. The major respiratory quinone is menaquinone-7 (MK-7). The type species is *Rhodocytophaga aerolata*.

**Description of *Rhodocytophaga aerolata* sp. nov.**

*Rhodocytophaga aerolata* (a.e.ro.la’ta. Gr. n. aer air; L. fem. part. adj. lata carried; N.L. fem. part. adj. aerolata airborne).

Displays the following characteristics in addition to those listed in the genus description. Cells are rod-shaped to filamentous (0.3–0.9 × 2.5–25.0 μm). Colonies on R2A agar are reddish pink and convex with entire margins. Optimum growth occurs at 30 °C and pH 7.0. The temperature and pH ranges for growth are 10–35 °C and pH 7.0–8.0. Hydrolyses CM-cellulose, DNA, starch and Tween 80, but not agar, casein, chitin, hypoxanthine, pectin or xanthine. Assimilates D-glucose, D-mannose, N-acetylglucosamine, maltose, L-rhamnose, sucrose, salicin and melibiose, but not D-ribose, D-mannitol, L-arabinose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, L-fucose, D-sorbitol, propionic acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, capric acid, adipic acid, malic acid, phenylacetic acid or L-proline. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase and N-acetyl-β-glucosaminidase activities, but negative for esterase lipase (C8), lipase (C14), trypsin, α-chymotrypsin, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase activities.

The detailed fatty acid composition of the type strain is given in Table 2. The DNA G+C content of the type strain is 45.5 mol%.

The type strain, 5416T-29T (=KACC 12507T =DSM 22190T), was isolated from an air sample from Suwon city, Republic of Korea.

**Acknowledgements**

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References


