Paenalcaligenes hominis gen. nov., sp. nov., a new member of the family Alcaligenaceae

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A beige-pigmented bacterium (strain CCUG 53761AT) was isolated from human blood from an 85-year-old man in Göteborg, Sweden. Comparative analysis of 16S rRNA gene sequences showed that this bacterium displayed <95% similarity to all described species of the genera of the family Alcaligenaceae. It grouped within the radiation of the genus Alcaligenes, but showed only 93.0–94.8% similarity to type strains of members of this genus (Alcaligenes faecalis subsp. parafaecalis, 94.8%; Alcaligenes faecalis subsp. faecalis, 94.2%; Alcaligenes faecalis subsp. phenolicus, 93.4%). This discrimination was supported by chemotaxonomic differences. The polyamine pattern consisted of the predominant compound putrescine, moderate amounts of spermidine and minor to trace amounts of spermine and cadaverine; 2-hydroxyputrescine was not detectable. The quinone system was ubiquinone Q-8 with minor amounts of Q-7. The polar lipid profile was composed of the major lipids diphosphatidylglycerol and phosphatidylethanolamine and moderate amounts of phosphatidylglycerol and an unknown phospholipid; minor lipids were also detected. The fatty acid profile, with large amounts of C16:0 and C17:0 cyclo and the absence of C12:0 2-OH as hydroxylated fatty acid, also differed significantly from those reported for Alcaligenes species. On the basis of these data, it is proposed that strain CCUG 53761AT represents a novel genus and species, for which the name Paenalcaligenes hominis gen. nov., sp. nov. is proposed. The type strain of Paenalcaligenes hominis is CCUG 53761AT = CCM 7698T.

The family Alcaligenaceae was proposed by De Ley et al. (1986) to accommodate the genera Bordetella and Alcaligenes. Subsequently, the genera Achromobacter, Advenella, Castellaniella, Pigm町phaga, Pusillimonas and Kerstersia have been assigned to the family (Yabuuchi et al., 1998; Blümel et al., 2001; Coenye et al., 2003a, 2005; Kämpfer et al., 2006; Stolz et al., 2005). The genera Oligella, Taylorella, Pelistega, Brackiella and Tetrathioibacter are closely related to the Alcaligenaceae but have not been formally assigned to this family (Rossau et al., 1987; Vandamme et al., 1998; Willems et al., 2002; Ghosh et al., 2005). Members of most of these genera have been isolated from various human, animal and environmental samples, but some of them (e.g. Pigm町phaga kulae, Tetrathioibacter species) appear to be truly environmental organisms, which have not been associated with human or animal diseases. Members of the family stain Gram-negative and are either strictly aerobic or facultatively anaerobic rods or cocccobacilli that are motile.

They possess oxidase and catalase. All grow well on complex media such as nutrient agar.

Strain CCUG 53761AT was isolated on blood agar at 37°C from human blood of an 85-year-old man in Göteborg, Sweden. Subcultivation was done on nutrient agar (NA; Oxoid) at 30°C for 24 h. Gram staining was performed with the standard procedure as described by Gerhardt et al. (1994). Cell morphology was examined with a Zeiss light microscope at ×1000 magnification, using cells that had been grown for 24 h at 30°C on NA.

The 16S rRNA gene was analysed as described by Kämpfer et al. (2003). DNA extraction was carried out using the GenElute Plant Genomic DNA kit (Sigma-Aldrich) according to the manufacturer’s instructions. The 16S rRNA gene was amplified by PCR using the primer pair 27F (5'-AGTATTGATCMTGCGCAG) and MR 1492R (5'-ACGYYTACCTGTTACGACTT) (Lane, 1991) and the following cycling conditions: 95°C for 3 min, 28 cycles of 94°C for 1 min, 57.3°C for 45 s and 72°C for 2 min and a final elongation step at 72°C for 15 min. The PCR product was purified with the QIAquick PCR
puriﬁcation kit (Qiagen) according to the manufacturer’s instructions. The PCR product was then sent to a sequencing service (Institute of Microbiology and Molecular Biology, Justus-Liebig-University Giessen, Giessen, Germany) and sequenced with standard sequencing primers for the 16S rRNA gene. Phylogenetic analysis was performed using the ARB software package (December 2007 version; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (July 2008 version; Pruesse et al., 2007). Trees were reconstructed using the maximum-likelihood method with fastDNAml (Olsen et al., 1994) and 50 % conservation filter (only alignment columns in which the frequency of the most abundant nucleotide is equal to or greater than 50 % are included in the calculation). Tree topology was further tested without ﬁlters. No signiﬁcant differences could be detected between these trees regarding the grouping of strain CCUG 53761T. Trees reconstructed with neighbour-joining and maximum-parsimony methods showed similar results (not shown). The almost-complete 16S rRNA gene sequence (1398 bp) of the strain was compared by distance calculations (distance options according to the Kimura-2 model) using the software package MEGA version 4.0 (Tamura et al., 2007). The results of these calculations indicated that the closest relatives of strain CCUG 53761T were Alcaligenes faecalis subsp. parafaecalis G1T (94.8 % similarity), Alcaligenes faecalis subsp. faecalis IAM 12369T (94.2 %) and Pusillimonas noertemannii BN9T (94.3 %). Lower sequence similarities (<94.2 %) were found with members of all other genera of the family Alcaligenaceae and with Alcaligenes faecalis subsp. phenolicus J1T (93.4 %). A maximum-likelihood tree is shown in Fig. 1; the close relationship to the genus Alcaligenes is obvious.

For extraction of quinones, polar lipids and polyamines, biomass was grown on PYE medium (0.3 % yeast extract, 0.3 % peptone from casein, pH 7.2). Extraction of respiratory quinones and polar lipids was carried out according to the procedure described by Tindall (1990a, b) and Altenburger et al. (1996). Polymyxins were extracted from cells harvested at the late exponential growth phase as reported by Busse & Auling (1988). HPLC analysis of quinones and polymyxins was carried out using the apparatus described by Stolz et al. (2007). Fatty acids were extracted and analysed according to Kämpfer & Kroppenstedt (1996). The polyamine pattern was composed of putrescine [104.1 μmol (g dry weight)−1], spermidine [3.2 μmol (g dry weight)−1], spermine [1.1 μmol (g dry weight)−1] and cadaverine [0.3 μmol (g dry weight)−1], but 2-hydroxyputrescine was not detectable. A polyamine pattern with the predominant compound putrescine and moderate to minor concentrations of spermidine is common for species of the Betaproteobacteria (Busse & Auling, 1988; 1990).

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses). The phylogenetic tree was constructed using the ARB software package (December 2007 version; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (July 2008 version; Pruesse et al., 2007). Tree building was performed using the maximum-likelihood method with fastDNAml (Olsen et al., 1994) and no conservation filter. Bar, 0.05 substitutions per nucleotide position.
Auling et al., 1991; Busse et al., 1992; Hamana & Takeuchi, 1998; Hamana et al., 2000, 2007). Lack of detectable amounts of 2-hydroxyputrescine is uncommon among species of the family Alcaligenaceae. All species of the family analysed for polyamines have been shown to contain significant amounts of 2-hydroxyputrescine (Busse & Auling, 1988; Hamana & Takeuchi, 1998; Stolz et al., 2005; Kämpfer et al., 2006), but Alcaligenes faecalis subsp. parafaecalis was reported to contain only trace amounts of this diamine. Hence, this polyamine pattern clearly distinguishes CCUG 53761\textsuperscript{T} from all recognized species of the family Alcaligenaceae, but further analyses of polyamines are needed to substantiate the uniqueness of this trait, since not all species of the family have been examined so far. Examination of close relatives of CCUG 53761\textsuperscript{T} is also desirable in order to collect knowledge about the stability of this trait within the proposed taxon. However, outside the family Alcaligenaceae, certain species of the class Betaproteobacteria have been reported to lack 2-hydroxyputrescine, such as Kingella denitrificans, Kingella kingae, Kingella oralis, Oxalobacter formigenes, Vitreoscilla beggiatoidea and Vitreoscilla stercoraria (Hamana et al., 2000), but these taxa are quite distant related to the family Alcaligenaceae, being placed on separate lines within the Betaproteobacteria (Moreira et al., 2000; Kirchhof et al., 2001). The quinone system consisted of ubiquinone Q-8 (93\%) and Q-7 (7\%), which is consistent with classification in the Betaproteobacteria (Yokota et al., 1992). The polar lipid profile was composed of the major compounds phosphatidylethanolamine and diphosphatidylglycerol, moderate amounts of phosphatidylglycerol and an unidentified phospholipid and minor amounts of three unidentified lipids and two unidentified aminolipids, one of which (AL2) corresponded to AL4 reported to be present in Castellaniella defragrans and Alcaligenes faecalis. This profile was less complex than those reported for species of the closely related genera Alcaligenes, Castellaniella and Pusillimonas (Stolz et al., 2005; Kämpfer et al., 2006) and contained characteristics useful for differentiation from these taxa (Fig. 2). The presence of the unidentified phospholipid PL (Fig. 2) and the lack of unidentified aminolipid AL1 present in Alcaligenes faecalis, Castellaniella defragrans, Castellaniella denitrificans and Pusillimonas notermannii (Stolz et al., 2005; Kämpfer et al., 2006) distinguish CCUG 53761\textsuperscript{T} from these related species. Lack of unidentified aminolipid AL2 allows specific differentiation of CCUG 53761\textsuperscript{T} from Pusillimonas notermannii.

The fatty acid profile of strain CCUG 53761\textsuperscript{T} is given in Table 1. The fatty acid profile is dominated by C\textsubscript{16:0}, C\textsubscript{17:0} cyclo and summed feature 3 (C\textsubscript{16:1}ω7c and/or C\textsubscript{15:0} iso 2-OH). The hydroxylated fatty acid C\textsubscript{12:0} 2-OH, present in all Alcaligenes species, was not found.

Results of physiological and biochemical characterization using methods that were described previously (Kämpfer et al., 1991) are given in the species description. Strain CCUG 53761\textsuperscript{T} was able to utilize some organic acids but no sugars or sugar-related compounds. On the basis of the phylogenetic and chemotaxonomic data, strain CCUG 53761\textsuperscript{T} is different from all other genera belonging to the family Alcaligenaceae (Tables 1 and 2). For this reason, a new genus and species are proposed, with the name Paenalcaligenes hominis gen. nov., sp. nov.

**Description of Paenalcaligenes gen. nov.**

*Paenalcaligenes* (Pa.en.al.ca.li’ge.nes. L. adv. paene nearly, almost; N.L. masc. n. *Alcaligenes* a bacterial genus name; N.L. masc. n. *Paenalcaligenes* almost *Alcaligenes*).

Cells are Gram-stain-negative, motile, short rods, 1.3–2.0 \(\mu\)m long and 0.2–0.8 \(\mu\)m wide. Oxidase-positive, showing an aerobic respiratory metabolism. Good growth occurs after 24 h incubation on nutrient agar at 25–30°C. The polyamine pattern is composed of the major compound putrescine and moderate amounts of spermidine. Minor amounts of spermine and cadaverine may be present, but 2-hydroxyputrescine is not detectable. The quinone system is ubiquinone Q-8 with minor amounts of Q-7. The polar lipid contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and the specific unidentified phospholipid PL (Fig. 2). The major fatty acids are C\textsubscript{16:0}, C\textsubscript{17:0} cyclo and summed feature 3 (C\textsubscript{16:1}ω7c and/or C\textsubscript{15:0} iso 2-OH); C\textsubscript{12:0} 2-OH is not detected. The type species is *Paenalcaligenes hominis*. The DNA G + C content of the type strain of the type species is 57 mol%.

**Description of Paenalcaligenes hominis sp. nov.**

*Paenalcaligenes hominis* (ho.mi.nis. L. gen. n. *hominis* of a man, of a human being, named because the type and only known strain is of human origin).
Table 1. Relative fatty acid compositions of the genera Paenalcaligenes gen. nov., Alcaligenes, Castellaniella, Pusillimonas, Achromobacter, Pigmentiphaga and Kerstersia

Genera: 1, Paenalcaligenes gen. nov.; 2, Alcaligenes (data from Coenye et al., 2003b); 3, Castellaniella (data from this study for C. defragrans DSM 12141T and C. denitrificans T4); 4, Pusillimonas (unless indicated, data from Stolz et al., 2005); 5, Achromobacter (Coenye et al., 2003a); 6, Pigmentiphaga (Blumel et al., 2001); 7, Kerstersia (Coenye et al., 2003b). Values are percentages of total fatty acids. tr, Trace amounts present; –, not detected/not reported. The presence or absence of the fatty acids in bold is of particular importance for differentiation among the listed genera; relative amounts of all listed fatty acids are also helpful for differentiation. Data for strain CCUG 53761A^T and strains of Castellaniella species were obtained in this study using cells grown on TSA (Oxoid) at 30 °C for 24 h prior to analysis. C. defragrans DSM 12141T also contained small amounts of C₁2:0 aldehyde (2.8%), C₁5:0 (0.4%), C₁7:0 (0.3%) and iso-C₁9:0 (0.3%). C. denitrificans T4 also contained small amounts of an unknown fatty acid (1.8%), C₁5:0 (1.4%), C₁7:0 (0.6%) and iso-C₁9:0 (0.7%).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₀:0</td>
<td>–</td>
<td>0–2.5</td>
<td>0–0.4</td>
<td>tr</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C₁₀:0 3-OH</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.9</td>
</tr>
<tr>
<td>C₁₂:0</td>
<td>3.0</td>
<td>tr–3.2</td>
<td>5.7–7.8</td>
<td>4.0</td>
<td>tr</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 2*</td>
<td>3.6</td>
<td>–</td>
<td>tr–0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C₁₂:0 2-OH</td>
<td>–</td>
<td>1.9–3.0</td>
<td>–</td>
<td>3.0</td>
<td>2.5–3.5</td>
<td>–</td>
<td>tr</td>
</tr>
<tr>
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<td>tr–2.2</td>
<td>0.5–1.0</td>
<td>–</td>
<td>1.1–5.6</td>
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<td>5.7</td>
</tr>
<tr>
<td>C₁₄:0 2-OH</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0–4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>C₁₄:0 3-OH</td>
<td>2.3</td>
<td>0–8.6</td>
<td>6.6–8.9</td>
<td>8.0</td>
<td>8.6–12.5</td>
<td>4.6</td>
<td>12.1</td>
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<tr>
<td>Summed feature 3*</td>
<td>24.0</td>
<td>10.3–30.4</td>
<td>11.7–36.0</td>
<td>tr</td>
<td>5.3–18.1</td>
<td>–</td>
<td>3.9</td>
</tr>
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<td>32.0</td>
<td>30.6–35.8</td>
<td>27.9–29.6</td>
<td>19.0</td>
<td>28.2–39.7</td>
<td>39.9</td>
<td>33.8</td>
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<td>C₁₇:0 cyclo</td>
<td>16.8</td>
<td>9.5–27.7</td>
<td>2.3–22.3</td>
<td>31.0</td>
<td>17.3–31.8</td>
<td>21.9</td>
<td>23.6</td>
</tr>
<tr>
<td>C₁₆:0 2-OH</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>tr</td>
<td>tr–2.4</td>
<td>4.8</td>
</tr>
<tr>
<td>C₁₆:0 3-OH</td>
<td>–</td>
<td>–</td>
<td>0–0.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>C₁₈:1ω7c</td>
<td>9.1</td>
<td>1.1–11.2</td>
<td>11.0–19.8</td>
<td>tr</td>
<td>1.8–5.2</td>
<td>9.8</td>
<td>10.7</td>
</tr>
<tr>
<td>C₁₈:0</td>
<td>2.6</td>
<td>tr–1.1</td>
<td>0–0.5</td>
<td>3.0</td>
<td>1.8–2.1</td>
<td>–</td>
<td>2.6</td>
</tr>
<tr>
<td>C₁₉:0 cyclo ω8c</td>
<td>0.7</td>
<td>tr</td>
<td>0–2.3</td>
<td>27.0</td>
<td>tr</td>
<td>12.2</td>
<td>2.0</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 contained C₁₂:0 aldehyde and/or an unknown fatty acid; summed feature 3 contained C₁₆:1ω7c and/or iso-C₁₅:0 2-OH.

The description is the same as for the genus, with the following additions. On NA, colonies are beige and circular with an entire margin. Grows at 30, 37 and 42 °C; no growth at 4 °C. L-Alanine p-nitroanilide (pNA) is hydrolysed, on the basis of the method described by Kämpfer et al. (1991). The following compounds are not hydrolysed: p-nitrophenyl (pNP) β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xylopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, l-aniline pNA, γ-L-glutamate pNA and L-proline pNA. The following compounds are used as sole sources of carbon on the basis of the method described by Kämpfer et al. (1991): acetate, cis- and trans-aconitate, pyruvate, fumarate, DL-3-hydroxybutyrate and DL-lactate. The following compounds are not assimilated: N-acetylgalactosamine, N-acetylgalactosamine, L-arabinose, L-arbutin, cellobiose, D-fructose, D-galactose, D-glucose, D-gluconate, maltose, D-mannose, α-melibiose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, D-xylene, adonitol, myo-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, adipate, azelate, 4-aminoobutyrate, citrate, glutarate, itaconate, L-malate, mesaconate, 2-oxoglutarate, propionate, suberate, L-alanine, β-alanine, L-aspartate, L-leucine, L-ornithine, L-proline, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate. No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol,
in sorbitol, l-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. Exhibits the chemotaxonomic characteristics listed in the genus description. In addition, two unidentified aminolipids (AL1, AL2) and three unidentified lipids (L1, L2, L3) are detectable.

The type strain is CCUG 53761AT (=CCM 7698T), isolated from human blood from an 85-year-old man in Göteborg, Sweden.

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