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

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A beige-pigmented bacterium (strain CCUG 53761A^T) 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. *paraecalis*, 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 C_{16:0} and C_{17:0} cyclo and the absence of C_{12:0} 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 53761A^T 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 53761A^T = CCM 7698^T.

The family *Alcaligenaceae* was proposed by De Ley *et al.* (1986) to accommodate the genera *Bordetella* and *Alcaligenes*. Subsequently, the genera *Achromobacter*, *Advenella*, *Castellaniella*, *Pigmentiphaga*, *Pusillimonas* and *Kerstersonia* 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 *Tetrathiobacter* 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. *Pigmentiphaga kullae*, *Tetrathiobacter* 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 coccobacilli that are motile.

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

Strain CCUG 53761A^T 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'-GAGTTTGATCMTGGCTCAG) and MR 1492R (5'-ACGGYTACCTTGTTACGACTT) (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

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 53761A^T is FN391024.

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 fastDNAmI (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 53761A^T. 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 53761A^T were *Alcaligenes faecalis* subsp. *para*faecalis G^T (94.8 % similarity), *Alcaligenes faecalis* subsp. *faecalis* IAM 12369^T

(94.2 %) and *Pusillimonas noertemannii* BN9^T (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* J^T (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). Polyamines were extracted from cells harvested at the late exponential growth phase as reported by Busse & Auling (1988). HPLC analysis of quinones and polyamines 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;

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 fastDNAmI (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. *para**faecalis* was reported to contain only trace amounts of this diamine. Hence, this polyamine pattern clearly distinguishes CCUG 53761A^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 53761A^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 beggiatoides* and *Vitreoscilla stercoraria* (Hamana *et al.*, 2000), but these taxa are quite distantly 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 noertemannii* (Stolz *et al.*, 2005; Kämpfer *et al.*, 2006) distinguish CCUG 53761A^T from these related species. Lack of unidentified aminolipid AL2 allows specific differentiation of CCUG 53761A^T from *Pusillimonas noertemannii*.

The fatty acid profile of strain CCUG 53761A^T is given in Table 1. The fatty acid profile is dominated by C_{16:0}, C_{17:0} cyclo and summed feature 3 (C_{16:1}ω7c and/or C_{15:0} iso 2-OH). The hydroxylated fatty acid C_{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 53761A^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

Fig. 2. Total polar lipid profile of strain CCUG 53761A^T after two-dimensional TLC and detection with molybdatophosphoric acid. PE, Phosphatidylethanolamine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; AL1–2, unknown aminolipids; PL, unknown phospholipid; L1–3, unknown polar lipids.

53761A^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 µm long and 0.2–0.8 µm 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_{16:0}, C_{17:0} cyclo and summed feature 3 (C_{16:1}ω7c and/or C_{15:0} iso 2-OH); C_{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 12141^T and *C. denitrificans* TJ4); 4, *Pusillimonas* (unless indicated, data from Stolz *et al.*, 2005); 5, *Achromobacter* (Coenye *et al.*, 2003a); 6, *Pigmentiphaga* (Blümel *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 12141^T also contained small amounts of C_{12:0} aldehyde (2.8 %), C_{15:0} (0.4 %), C_{17:0} (0.3 %) and iso-C_{19:0} (0.3 %). *C. denitrificans* TJ4 also contained small amounts of an unknown fatty acid (1.8 %), C_{15:0} (1.4 %), C_{17:0} (0.6 %) and iso-C_{19:0} (0.7 %).

Fatty acid	1	2	3	4	5	6	7
C _{10:0}	—	0–2.5	0–0.4	tr	—	—	—
C _{10:0} 3-OH	—	—	—	—	—	2.9	—
C_{12:0}	3.0	tr–3.2	5.7–7.8	4.0	tr	—	—
Summed feature 2*	3.6	—	tr–0.1	—	—	—	—
C_{12:0} 2-OH	—	1.9–3.0	—	3.0	2.5–3.5	—	tr
C _{14:0}	2.2	tr–2.2	0.5–1.0	—	1.1–5.6	—	5.7
C_{14:0} 2-OH	—	—	—	—	0–4.0	4.0	4.3
C _{14:0} 3-OH	2.3	0–8.6	6.6–8.9	8.0	8.6–12.5	4.6	12.1
Summed feature 3*	24.0	10.3–30.4	11.7–36.0	tr	5.3–18.1	—	3.9
C _{16:0}	32.0	30.6–35.8	27.9–29.6	19.0	28.2–39.7	39.9	33.8
C_{17:0} cyclo	16.8	9.5–27.7	2.3–22.3	31.0	17.3–31.8	21.9	23.6
C _{16:0} 2-OH	—	—	—	tr	tr–2.4	4.8	—
C _{16:0} 3-OH	—	—	0–0.4	—	—	—	—
C _{18:1} ω7c	9.1	1.1–11.2	11.0–19.8	tr	1.8–5.2	9.8	10.7
C_{18:0}	2.6	tr–1.1	0–0.5	3.0	1.8–2.1	—	2.6
C_{19:0} cyclo ω8c	0.7	tr	0–2.3	27.0	tr	12.2	2.0

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI System. Summed feature 2 contained C_{12:0} aldehyde and/or an unknown fatty acid; summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 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

Table 2. Chemotaxonomic characteristics that distinguish strain CCUG 53761A^T from the type strains of the type species of *Alcaligenes*, *Castellaniella* and *Pusillimonas*

Strains: 1, CCUG 53761A^T; 2, *Alcaligenes faecalis* DSM 30030^T (data from Stolz *et al.*, 2005); 3, *Castellaniella defragrans* NKNTAU^T (Kämpfer *et al.*, 2006); 4, *Pusillimonas noertemannii* BN9^T (Stolz *et al.*, 2005). Lipids are designated according to Fig. 2 and these may vary from the designations used by Stolz *et al.* (2005) and Kämpfer *et al.* (2006).

Characteristic	1	2	3	4
Polyamine profile				
2-Hydroxyputrescine	—	+	+	+
Polar lipid profile				
PL	+	—	—	—
AL2	+	+	+	—
AL1	—	+	+	+

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-glutamyl 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-acetylglucosamine, 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-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, adipate, azelate, 4-aminobutyrate, 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,

inositol, 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 53761A^T (=CCM 7698^T), isolated from human blood from an 85-year-old man in Göteborg, Sweden.

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