Candidimonas nitroreducens gen. nov., sp. nov. and *Candidimonas humi* sp. nov., isolated from sewage sludge compost

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Two bacterial strains (SC-089^T and SC-092^T) isolated from sewage sludge compost were characterized by using a polyphasic approach. The isolates were Gram-negative short rods, catalase- and oxidase-positive, and showed good growth at 30 °C, at pH 7 and with 1 % (w/v) NaCl. Ubiquinone 8 was the major respiratory quinone, and phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol were amongst the major polar lipids. On the basis of 16S rRNA gene sequence analysis, the strains were observed to be members of the family *Alcaligenaceae*, but could not be identified as members of any validly described genus. The low levels of 16S rRNA gene sequence similarity to other recognized taxa, together with comparative analysis of phenotypic traits and chemotaxonomic markers, supported the proposal of a new genus within the family *Alcaligenaceae*, for which the name *Candidimonas* gen. nov. is proposed. Strains SC-089^T and SC-092^T, which shared 99.1 % 16S rRNA gene sequence similarity, could be differentiated at the phenotypic level, and DNA–DNA hybridization results supported their identification as representing distinct species. The names proposed for these novel species are *Candidimonas nitroreducens* sp. nov. (type strain, SC-089^T=LMG 24812^T=CCUG 55806^T) and *Candidimonas humi* sp. nov. (type strain, SC-092^T=LMG 24813^T=CCUG 55807^T).

Two bacterial strains, designated SC-089^T and SC-092^T, were isolated from sewage sludge compost during a study on the diversity of culturable bacteria from final composts (Vaz-Moreira *et al.*, 2008). As discussed below, these isolates shared about 99 % 16S rRNA gene sequence similarity and were observed to be members of the family *Alcaligenaceae*. Among the closest neighbours of strains SC-089^T and SC-092^T were members of the genera *Parapusillimonas*, *Achromobacter*, *Bordetella*, *Castellaniella*, *Pigmentiphaga* and *Pusillimonas*, with *Parapusillimonas granuli*, *Pigmentiphaga kullae* and *Bordetella petrii* as nearest neighbours. To test the hypothesis that strains SC-089^T and SC-092^T could be members of a new genus, these strains were compared with the type strains of the type species of the above genera, as well as with the type

strain of the closest related species within each genus. The only exception was made for the type species *Bordetella pertussis*, which, given its potential hazardous nature, was substituted by *Bordetella bronchiseptica*, with which the type strains studied here shared identical 16S rRNA gene sequences. Thus, strains SC-089^T and SC-092^T were compared with *Parapusillimonas granuli* LMG 24012^T, *Pigmentiphaga kullae* CCUG 47266^T, *B. petrii* CCUG 43448^T, *B. bronchiseptica* LMG 1232^T, *Achromobacter xylosoxidans* LMG 1863^T, *Achromobacter denitrificans* LMG 1231^T, *Castellaniella defragrans* LMG 18538^T, *Castellaniella denitrificans* CCUG 39541^T and *Pusillimonas noertemannii* DSM 10065^T, which were supplied by the respective culture collections. Unless stated otherwise, the organisms were cultivated on plate count agar (PCA; Pronadisa), incubated at 30 °C and stored at –80 °C in nutritive broth with 15 % (v/v) glycerol. Colony and cell morphology, Gram staining, cytochrome *c* oxidase and catalase tests, and motility were analysed based on the

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SC-089^T and SC-092^T are FN556191 and FN556192, respectively.

methodologies of Murray *et al.* (1994) and Smibert & Krieg (1994). Additional phenotypic characterization was based on the methods described previously (Vaz-Moreira *et al.*, 2007). Biochemical and nutritional tests were performed by using the commercial kits API 20E, API 20NE, API ZYM and API ID 32GN (bioMérieux) following the manufacturer's instructions. The Voges–Proskauer test was assayed in methyl red and Voges–Proskauer medium (Oxoid), incubated at 30 °C for 48 h. Simmons citrate was tested on Simmons citrate agar (Pronadisa) incubated for 7 days at 30 °C. Urease activity was assayed as described by Smibert & Krieg (1994).

After 48 h incubation at 30 °C on PCA, strains SC-089^T and SC-092^T formed convex white colonies. Growth was optimal at 30 °C, at pH 7 and in the presence of 1 % (w/v) NaCl and was supported by different amino acids and organic acids. The nucleotide sequence of the 16S rRNA gene was determined after PCR amplification of total DNA extracts as described previously (Ferreira da Silva *et al.*, 2007). The 16S rRNA gene sequence was compared with others available in GenBank databases by using the FASTA package from EMBL-EBI. Phylogenetic analysis was conducted using the BioNumerics software package (version 6.0; Applied Maths). Sequence similarity was estimated based on the model of Jukes & Cantor (1969) and the dendrogram was created with the neighbour-joining method. The maximum-parsimony and maximum-likelihood methods were used to assess tree stability. Non-homologous and ambiguous nucleotide positions were excluded from the calculations. Strains SC-089^T and SC-092^T shared 99.1 % 16S rRNA gene sequence similarity, but shared lower levels with *Parapusillimonas granuli* LMG

24012^T (97.8 and 97.4 %, respectively), *Pigmentiphaga kullae* CCUG 47266^T (97.1 and 97.3 %) and *B. petrii* CCUG 43448^T (both 96.9 %), which represented their nearest phylogenetic neighbours (Fig. 1). Either both strains SC-089^T and SC-092^T or one of them showed lower 16S rRNA gene sequence similarity to members of other related species: respectively 97.0 and 96.6 % with *A. xylosoxidans* LMG 1863^T, 96.9 and 96.8 % with *A. denitrificans* LMG 1231^T, 96.3 and 95.8 % with *B. bronchiseptica* LMG 1232^T, 96.5 and 96.0 % with *C. defragrans* LMG 18538^T, 96.6 and 96.1 % with *C. denitrificans* LMG 25533^T, and 96.2 and 95.9 % with *Pusillimonas noertemannii* DSM 10065^T. The two novel strains represented a distinct line of descent in the family *Alcaligenaceae* and their phylogenetic position was supported by a high bootstrap value and by the different treeing algorithms used.

DNA–DNA hybridizations were performed between strains SC-089^T and SC-092^T, and with *Pigmentiphaga kullae* CCUG 47266^T and *Parapusillimonas granuli* LMG 24012^T. DNA was extracted from 0.25–0.5 g (wet weight) cells as described by Pitcher *et al.* (1989). DNA–DNA hybridizations were performed with photobiotin-labelled probes in microplate wells (Ezaki *et al.*, 1989), by using an HTS7000 Bio Assay Reader (Perkin Elmer) for fluorescence measurements. The hybridization temperature was 47 °C. Reciprocal experiments were performed for every pair of strains and levels of relatedness differed by between 1 and 7 %. The level of DNA–DNA relatedness between strains SC-089^T and SC-092^T was 41 %; levels of relatedness with the other type strains investigated were 30 % or lower (data not shown).

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequence analysis, showing the relationship of strains SC-089^T and SC-092^T with members of the family *Alcaligenaceae*. The tree was generated by the neighbour-joining method and *Zoogloea ramigera* ATCC 19324 was used as outgroup. Bootstrap values, generated from 1000 resamplings, at or above 50 % are indicated at branch points. Closed circles indicate branches also recovered by the maximum-likelihood method. Bar, 1 substitution per 100 nucleotide positions.

Fatty acid methyl esters were analysed after an incubation period of 24 or 48 h (*C. defragrans* LMG 18538^T and *Pusillimonas noertemanni* DSM 10065^T) at 28 °C. A loopful of well-grown cells was harvested and fatty acid methyl esters were prepared as described previously (Vandamme *et al.*, 1992), and separated and identified by using the Sherlock Microbial Identification System (version 3.1; MIDI Inc.). The polar lipid composition was determined as described previously (Manaia *et al.*, 2004) (Fig. 2).

Determination of the G + C content of the genomic DNA and of the respiratory quinones was performed as described previously (Vaz-Moreira *et al.*, 2007) by using, respectively, the methods of Mesbah *et al.* (1989) and Tindall (1989). The respiratory quinone was ubiquinone 8. The DNA G + C content of strains SC-089^T and SC-092^T was 64 and 65 mol%, respectively. These chemotaxonomic traits confirm the affiliation of strains SC-089^T and SC-092^T to the family *Alcaligenaceae* (Vancanneyt *et al.*, 1995; Blümel *et al.*, 2001; von Wintzingerode *et al.*, 2001; Busse &

Auling, 2005; Stolz *et al.*, 2005), although the unique phylogenetic position suggests that they are most appropriately allocated to a new genus.

At the phenotypic level, strains SC-089^T and SC-092^T could also be distinguished from the type strains of the type species of related genera as well as from the type strains of the most closely related species (Table 1). For example, strains SC-089^T and SC-092^T could be distinguished from the members of the other genera examined in this study based on the absence of motility (present in members of the genera *Achromobacter*, *Pigmentiphaga*, *Castellaniella*, *Pusillimonas* and *Parapusillimonas*), inability to assimilate L-alanine (observed in members of the genera *Achromobacter*, *Bordetella*, *Castellaniella* and *Parapusillimonas*) and L-proline (also absent in members of the genera *Pigmentiphaga* and *Pusillimonas*), and the ability to assimilate 3-hydroxybenzoic acid (absent in members of the genus *Bordetella*) and several other organic acids and sugars. These differential phenotypic characteristics, along with their unique phylogenetic position, suggest that

Fig. 2. Polar lipid patterns of strains SC-089^T and SC-092^T and related members of the family *Alcaligenaceae*. PE, Phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PL, APL, GL and AL, respectively, unidentified phospholipid, aminophospholipid, glycolipid and aminolipid.

Table 1. Differential characteristics between strains SC-089^T and SC-092^T and the type strains of closely related species

Strains: 1, SC-089^T; 2, SC-092^T; 3, *Pigmentiphaga kullae* CCUG 47266^T; 4, *Bordetella petrii* CCUG 43448^T; 5, *Bordetella bronchiseptica* LMG 1232^T; 6, *Achromobacter xylosoxidans* LMG 1863^T; 7, *Achromobacter denitrificans* LMG 1231^T; 8, *Castellaniella defragrans* LMG 18538^T; 9, *Castellaniella denitrificans* CCUG 39541^T; 10, *Pusillimonas noertemannii* DSM 10065^T; 11, *Parapusillimonas granuli* LMG 24012^T. Data are from the present study except where indicated otherwise. w, Weakly positive; NA, not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Motility	—	—	+ ^{xa}	— ^b	+ ^c	+ ^d	+ ^d	+ ^e	+ ^e	+ ^f	+ ^g
Simmons citrate	—	—	—	+	—	+	—	+	—	—	—
Urease	—	—	—	—	+	—	—	—	—	—	+
Assimilation of:											
Glucose	—	—	+	—	—	+	—	—	—	w	+
D-Mannose	—	—	+	—	—	w	—	—	—	—	—
L-Alanine	—	—	—	+	+	+	+	+	+	—	+
L-Proline	—	—	—	+	—	+	+	+	+	—	+
L-Serine	—	—	—	+	—	+	—	+	—	—	+
Adipate	+	+	+	+	+	+	+	—	—	—	+
Caprate	—	—	—	—	w	+	w	w	w	—	—
3-Hydroxybenzoic acid	+	+	+	—	—	—	+	+	+	+	+
Itaconic acid	+	+	w	+	+	+	+	—	+	—	+
Lactic acid	+	+	—	+	+	+	+	+	+	—	+
Phenyl acetate	+	+	—	—	+	+	+	w	—	—	+
Propionic acid	+	+	—	+	+	+	+	+	+	+	+
Sodium acetate	+	+	—	+	+	+	+	+	+	+	+
Suberic acid	—	—	—	+	—	+	+	—	—	—	—
Valeric acid	+	+	—	+	+	+	+	+	+	—	+
DNA G+C content (mol%)	63.9	65.2	68.5 ^a	63.8 ^b	67–69 ^{c†}	66.9–69.8 ^{d†}	63.9–68.9 ^{d†}	66.9 ^{h†}	NA	61.8 ^{f†}	67.9 ^g
Source of isolation of the type strain	Sewage sludge compost	Sewage sludge compost	Soil	Dechlorinating bioreactor/clinical sources	Respiratory tract of animals	Mostly clinical isolates	Soil/clinical isolates	Alkenoic monoterpenes	Anaerobic sludge digester	Water	Anaerobic sludge blanket reactor

*Data from: *a*, Blümel *et al.* (2001); *b*, von Wintzingerode *et al.* (2001); *c*, Sanden & Weyant (2005); *d*, Busse & Auling (2005); *e*, Kämpfer *et al.* (2006); *f*, Stolz *et al.* (2005); *g*, Kim *et al.* (2010); *h*, Foss *et al.* (1998).

†Determined by *T_m*.

strains SC-089^T and SC-092^T should be most appropriately allocated to a new genus.

Cellular fatty acid composition also allowed strains SC-089^T and SC-092^T to be differentiated from the type strains of related species (Table 2). The predominant fatty acids common to strains SC-089^T and SC-092^T were summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I), C_{16:0} and C_{18:1}ω7c. The two organisms differed in the abundance of cyclo-C_{17:0} and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and in the presence of iso-C_{17:0} 3-OH. The pattern of fatty acids of strains SC-089^T and SC-092^T was different from those of the strains examined in at least two components that differed by >5%. The presence of C_{16:0} 2-OH and C_{16:1} 2-OH was also distinctive, these components only being present in strains SC-089^T and SC-092^T and in *Pigmentiphaga kullae* LMG 21665^T. However, C_{12:0} 2-OH was not detected in *Pigmentiphaga kullae* LMG 21665^T.

Analysis of the pattern of polar lipids also distinguished strains SC-089^T and SC-092^T from members of the closely related genera (Fig. 2). Although phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol were present in members of the other genera, their proportions, along with the presence/absence of unknown phospholipids and aminolipids, were distinctive characteristics among the taxa under study. *Pigmentiphaga kullae* CCUG 47266^T presented the most differentiated polar lipid pattern, with a low

proportion of diphosphatidylglycerol and the absence of most of the unknown phospholipids and amino(phospho)lipids otherwise noted. The absence of unknown aminolipid AL2 permitted us to distinguish between strains SC-089^T and SC-092^T and members of other related genera, as it constitutes a major lipid in *Pusillimonas noertemannii* DSM 10065^T, and is present in low proportions in *A. xylosoxidans* LMG 1863^T. Additionally, the exclusive presence of unknown aminophospholipids APL2, APL3 and APL4 in strains SC-089^T and SC-092^T contributed to distinguishing these organisms from related genera (Table 3).

Distinction of genera of the family *Alcaligenaceae* on the basis of phenotypic characteristics is not possible, as demonstrated in previous studies, namely for the descriptions of the genera *Pigmentiphaga*, *Pusillimonas* and *Parapusillimonas* (Blümel *et al.*, 2001; Stolz *et al.*, 2005; Kim *et al.*, 2010), which do not refer to any distinctive phenotypic trait. In fact, a literature review of studies of the phenotypic description of members of the genera *Achromobacter*, *Bordetella*, *Castellaniella*, *Pusillimonas*, *Pigmentiphaga* and *Parapusillimonas* shows that, among a group of >50 tests of carbon-source assimilation, enzyme production/activity and other physiological traits, these characteristics are variable within each genus and/or are not differential among these genera (Kerstens *et al.*, 1984; Foss *et al.*, 1998; Vandamme

Table 2. Fatty acid composition of strains SC-089^T and SC-092^T and the type strains of closely related species

Strains: 1, SC-089^T; 2, SC-092^T; 3, *Pigmentiphaga kullae* LMG 21665^T; 4, *Bordetella bronchiseptica* LMG 1232^T; 5, *Bordetella petrii* CCUG 43448^T; 6, *Achromobacter xylosoxidans* LMG 1863^T; 7, *Achromobacter denitrificans* LMG 1231^T; 8, *Castellaniella defragrans* LMG 18538^T; 9, *Castellaniella denitrificans* CCUG 39541^T; 10, *Pusillimonas noertemannii* DSM 10065^T; 11, *Parapusillimonas granuli* LMG 24012^T. Those fatty acids for which the amount for all taxa was <1% are not included. Therefore, the percentages may not add up to 100%. tr, Trace amount (<1%); ND, not detected.

Fatty acid	1*	2	3†	4	5	6	7	8	9	10‡	11§
C _{10:0} 2-OH	1.7	tr	ND	ND	ND	ND	ND	ND	ND	ND	ND
C _{12:0}	tr	tr	ND	tr	tr	tr	tr	4.8	6.5	8.2	ND
C _{12:0} 2-OH	2.4	4.0	ND	2.7	2.5	3.9	3.4	ND	ND	6.3	ND
C _{14:0}	ND	ND	ND	4.8	5.4	1.7	5.1	tr	tr	ND	ND
C _{14:0} 2-OH	ND	ND	4.6	ND	tr	3.4	ND	ND	ND	ND	ND
SF2	20.4	16.7	6.8	7.6	8.4	10.6	10.7	6.4	8.6	22.7	7.4
SF3¶	9.7	25.2	9.6	26.3	20.0	16.0	22.4	21.7	24.7	3.9	28.5
C _{16:0}	25.9	25.1	28.4	34.1	32.2	33.8	30.2	30.4	31.6	10.3	33.2
C _{17:0} cyclo	17.9	6.2	17.1	10.6	16.0	19.6	15.2	15.8	13.0	18.9	18.2
C _{16:1} 2-OH	1.5	3.6	1.1	ND	ND	ND	ND	ND	ND	ND	ND
C _{16:0} 2-OH	1.9	1.6	2.9	ND	ND	ND	ND	ND	ND	ND	ND
C _{18:1} ω7c	10.8	13.1	17.5	10.2	9.8	5.6	7.8	17.4	12.7	2.6	11.3
C _{18:0}	2.4	3.6	1.1	2.4	2.6	1.5	1.5	tr	tr	1.4	tr
C _{19:0} cyclo ω8c	1.3	ND	5.0	ND	tr	tr	tr	tr	tr	10.1	tr

*Strain SC-089^T also contains 3.1% iso-C_{17:0} 3-OH.

†*Pigmentiphaga kullae* LMG 21665^T also contains 3.4% C_{10:0} 3-OH and 2.4% C_{18:1} 2-OH.

‡*Pusillimonas noertemannii* DSM 10065^T also contains 2.9% C_{19:0} 10 methyl, 4.2% C_{16:0} 3-OH and 5.8% C_{18:1} 2-OH.

§Data from Kim *et al.* (2010).

||SF2, Summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I).

¶SF3, Summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH).

Table 3. Qualitative comparison of the polar lipid profiles of different genera of the family *Alcaligenaceae*, according to criteria used by Stolz *et al.* (2005)

Genera: 1, *Candidimonas* gen. nov.; 2, *Achromobacter*; 3, *Bordetella*; 4, *Castellaniella*; 5, *Pigmentiphaga*; 6, *Pusillimonas*; 7, *Parapusillimonas*. PE, Phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; APL and AL, respectively, unidentified aminophospholipid and aminolipid.

Polar lipid	1	2	3	4	5	6	7
PE	+	++	++	++	+	+	++
PG	+	++	++	++	+	+	+
DPG	+	+	++	++	±	+	++
APL1	+	–	–	–	–	–	+
APL2	+	–	–	–	–	–	–
APL3	+	–	–	–	–	–	–
APL4	+	–	–	–	–	–	–
APL5	–	–	+	–	–	–	–
APL6	–	–	+	–	–	–	–
AL1	–	+	+	+	–	+	–
AL2	–	+	–	–	–	++	–

et al., 1995, 1996; Weyant *et al.*, 1995; Blümel *et al.*, 2001; von Wintzingerode *et al.*, 2001; Coenye *et al.*, 2003; Busse & Auling, 2005; Stolz *et al.*, 2005; Kämpfer *et al.*, 2006; Yoon *et al.*, 2007; Liu *et al.*, 2008; Chen *et al.*, 2009; Kim *et al.*, 2009, 2010; Lee *et al.*, 2010; Srinivasan *et al.*, 2010). In this respect, comparison of the type strains of the type species and of closely related species, as performed here and by Kim *et al.* (2010) in the description of the genus *Parapusillimonas*, allows an objective comparison of related taxa, useful for future identification or comparative assays. Due to the limitations mentioned above, some authors have supported the distinction of genera of the family *Alcaligenaceae* on the basis of phylogenetic or chemotaxonomic markers such as 16S rRNA gene sequence signature nucleotides or the qualitative comparison of polar lipid patterns. Accordingly, strains SC-089^T and SC-092^T were compared with the type strains of their most closely related species and the type strains of type species of related genera on the basis of such criteria (Tables 3 and 4; Fig. 2). The two approaches allowed the distinction of these novel strains from related genera, supporting the proposal of a new genus.

Strains SC-089^T and SC-092^T were observed to be phenotypically distinct, namely in their ability to reduce nitrate, grow anaerobically, oxidize glucose and assimilate some carbon sources (potassium 2-ketogluconate, 4-hydroxybenzoic acid, trisodium citrate, malate and potassium gluconate). Additionally, the two strains differed in their cellular fatty acid profile and DNA G+C content. These differences, together with a 16S rRNA gene sequence similarity of 99.1 % and low level of DNA–DNA relatedness, suggest that strains SC-089^T and SC-092^T represent two novel species of a new genus, for which we propose the

names *Candidimonas nitroreducens* gen. nov., sp. nov. and *Candidimonas humi* sp. nov., respectively.

Description of *Candidimonas* gen. nov.

Candidimonas [Can.di.di.mo'nas. L. adj. *candidus* -a -um white; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Candidimonas* a unit (rod) that produces white colonies].

Cells are non-spore-forming, Gram-negative, non-motile, curved short rods. Catalase- and cytochrome *c* oxidase-positive. Mesophilic. Chemo-organotrophic with aerobic respiratory metabolism. Amino acids and organic acids are used as sole carbon sources. Anaerobic growth, nitrate reduction, glucose oxidation and the assimilation of some organic acids as sole carbon sources are variable characteristics of the genus. The respiratory quinone is ubiquinone 8 and the DNA G+C content is 64–65 mol%. Major fatty acids are summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I), C_{16:0} and C_{18:1ω7c}. The polar lipids present are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and four unidentified aminophospholipids. Phylogenetically, belongs to the family *Alcaligenaceae*. The type species is *Candidimonas nitroreducens*.

Description of *Candidimonas nitroreducens* sp. nov.

Candidimonas nitroreducens (ni.tro.re.du'cens. N.L. n. *nitras* -atis nitrate; N.L. pref. *nitro*- pertaining to nitrate; L. part. adj. *reducens* leading back, bringing back and, in chemistry, converting to a different oxidation state; N.L. part. adj. *nitroreducens* reducing nitrate).

Colonies are white, circular (~1 mm diameter) and convex on PCA after 48 h incubation. Cells are coccobacilli (0.53 ± 0.11 µm). Growth occurs at 15–40 °C, at pH 5–8 and in the presence of up to 3 % (w/v) NaCl [optima at about 30 °C, pH 7 and 1 % (w/v) NaCl]. Under anaerobiosis, growth is slow and weak and nitrate is reduced to nitrite. Simmons citrate is not utilized. H₂S, indole and acetoin are not produced. Aesculin is not hydrolysed. Glucose is oxidized (API 20E), but D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose are not. Glucose is not fermented. Produces acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase, but not arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, gelatinase, urease, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. The following sole carbon sources are assimilated: adipate, phenyl acetate, itaconic acid, sodium acetate, lactic acid, 3-hydroxybenzoic acid, propionic acid, valeric acid, 3-hydroxybutyric acid and 4-hydroxybenzoic acid. Assimilation of malate and potassium gluconate is weak. The following sole carbon sources are not assimilated:

Table 4. Pattern of 16S rRNA signature nucleotides that distinguish different genera within the family *Alcaligenaceae*, selected as proposed by Blümel *et al.* (2001)

Genera: 1, *Candidimonas* gen. nov.; 2, *Achromobacter*; 3, *Bordetella*; 4, *Castellaniella*; 5, *Pigmentiphaga*; 6, *Pusillimonas*; 7, *Parapusillimonas*. Positions given are relative to *Escherichia coli* numbering and this analysis included the type strains of recognized species belonging to each of the indicated genera. Bold type indicates signature nucleotides different from those of the novel type strains, SC-089^T and SC-092^T. GenBank accession numbers are as indicated in Fig. 1.

Position	1	2	3	4	5	6	7
43:399	G:C	G:C	G:C	G:C	C:G	G:C	G:C
82:87	C:G	C:G	C:G	G:C	C:G	A:U	C:G
83	U	U	U	C	U	U*	U
85	C	C	C	U	C	G*	C
123:238	U:A	U:A	U:A	U:A	C:G†	U:A	U:A
200:217	G:C	A:U	G:C	G:C	G:C	G:C	G:C
207:212	U:A	U:A	C:G	U:A	U:A	C:G	U:A
208	G	G	U	G	G	C*	G
209	C	C	U	C	C	U*	C
210	A	A	C	A	A	U*	A
211	A	A	G	A	A	C*	A
412	U	U	U	U	A	U	U
624:616	C:G	U:A‡	C:G§	C:G§	C:G	C:G*	C:G
673:717	A:U	G:C‡	G:C	A:U	G:C	A:U	A:U
1060:1197	U:A	U:A	U:A	U:A	C:G	U:A	U:A
1030	C	C	C	C	A	C	C
1137	A	A	A	G	A	A	A
1256	C	U	U	U¶	C	U	U
1283	C	U	C	U	C	U*	C
1308	C	C	C	C	U	U	U
1329	G	G	G	G	A	A	A

*In *Pusillimonas ginsengisoli*, G is observed in positions 83 and 208, U in position 87, C in positions 209 and 1283, A in positions 211 and 412 and the pair A:U in position 624:616.

†In *Pigmentiphaga litoralis*, a U:A pair is observed in this position.

‡In *A. insolitus*, C:G and A:U pairs are observed in positions 624:616 and 673:717, respectively.

§In *B. avium*, *B. hinzii* and *C. defragrans*, a U:A pair is observed.

||C in *B. petrii*.

¶A in *C. caeni*.

D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, citrate, L-rhamnose, D-ribose, inositol, sucrose, suberic acid, sodium malonate, L-alanine, potassium 5-ketogluconate, glycogen, L-serine, salicin, melibiose, L-fucose, D-sorbitol, capric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate and L-proline. The predominant fatty acids are those listed in the genus description and C₁₇:0 cyclo.

The type strain, SC-089^T (=CCUG 55806^T=LMG 24812^T), was isolated from sewage sludge compost. The DNA G+C content of the type strain is 64 mol%.

Description of *Candidimonas humi* sp. nov.

Candidimonas humi (hu'mi. L. n. *humus* earth, soil and, in earth sciences or agriculture, humus; L. gen. n. *humi* of the soil, of the humus).

Colonies are white, circular (~1 mm diameter), convex and rough on PCA after 48 h incubation. Cells are coccobacilli (0.50±0.09 µm). Growth occurs at 15–40 °C, at pH 5–8 and in the presence of up to 3% (w/v) NaCl [optima about 30 °C, pH 7 and 1% (w/v) NaCl]. Unable to grow under anaerobiosis or to reduce nitrate. Simmons citrate is not utilized. H₂S, indole and acetoin are not produced. Aesculin is not hydrolysed. Glucose is not fermented. Produces acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase and α-chymotrypsin, but not arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, gelatinase, urease, lipase (C14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. The following sole carbon sources are assimilated: adipate, citrate, phenyl acetate, itaconic acid, sodium acetate,

lactic acid, 3-hydroxybenzoic acid, propionic acid, valeric acid, trisodium citrate, potassium 2-ketogluconate and 3-hydroxybutyric acid. The carbon sources D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, malate, potassium gluconate, L-rhamnose, D-ribose, inositol, sucrose, suberic acid, sodium malonate, L-alanine, potassium 5-ketogluconate, glycogen, L-serine, salicin, melibiose, L-fucose, D-sorbitol, capric acid, L-histidine, 4-hydroxybenzoic acid and L-proline are not assimilated. The predominant fatty acids are those listed in the genus description and summed feature 3 (C₁₆:1 ω 7c or iso-C₁₅:0 2-OH or both).

The type strain, SC-092^T (=CCUG 55807^T=LMG 24813^T), was isolated from sewage sludge compost. The DNA G+C content of the type strain is 65 mol%.

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