Humibacter albus gen. nov., sp. nov., isolated from sewage sludge compost

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A bacterial strain isolated from sewage sludge compost, strain SC-083^T, was characterized. The isolate was a motile, Gram-positive, short rod, forming coryneform V-shaped cells during the early stages of growth. The organism was strictly aerobic and able to grow between 22 and 36 °C and between pH 5.5 and 8.0. The predominant fatty acids were cyclohexyl-$C_{17:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$, the major respiratory quinones were menaquinone 11 (MK-11) and 12 (MK-12), and the genomic DNA G+C content was 68 mol%. The peptidoglycan contained the diagnostic diamino acids ornithine and 2,4-diaminobutyric acid and was of acetyl type. The 16S rRNA gene sequence analysis indicated that this isolate belongs to the family *Microbacteriaceae* with the type strains of the species *Leifsonia xyli* (96 % gene sequence similarity), *Leifsonia shinshuensis* (96 %), *Leifsonia naganoensis* (95 %), *Leifsonia aquatica* (95 %), *Agromyces ramosus* (95 %) and *Curtobacterium citreum* (95 %) among the closest phylogenetic neighbours. The phylogenetic analysis and phenetic characteristics support the proposal of a new genus and a novel species, with the name *Humibacter albus* gen. nov., sp. nov. The type strain of *Humibacter albus* is SC-083^T (=DSM 18994^T =CCUG 54538^T =LMG 23996^T).

The family *Microbacteriaceae* comprises 22 genera with validly published names (Euzéby, 1997). The cell wall composition and respiratory quinones represent important chemotaxonomic distinctive traits within this family (Takeuchi & Hatano, 1998; Evtushenko *et al.*, 2000; Kämpfer *et al.*, 2000; Han *et al.*, 2003; Manaia *et al.*, 2004). In general, members of the *Microbacteriaceae* do not exhibit relevant variations in fatty acid composition, with the components anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$ representing 60–70 % of the total, even when different growth conditions or extraction and analytical methods are used (Suzuki *et al.*, 1997, 1999; Takeuchi & Hatano, 1998; Kämpfer *et al.*, 2000; Han *et al.*, 2003; Manaia *et al.*, 2004; Yoon *et al.*, 2006). Cyclohexyl fatty acids have been described in some members of this family (Qiu *et al.* 2007; Aizawa *et al.*, 2007); nevertheless, the chemotaxonomic and phylogenetic significance of this cell membrane component in these organisms is not clearly ascertained at the moment.

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the isolate SC-083^T is AM494541.

This paper describes the characterization of a member of the family *Microbacteriaceae*, strain SC-083^T, isolated from municipal sewage sludge compost (Vaz-Moreira *et al.*, 2008). The compost is produced in a windrow digester from anaerobically digested sludge of a municipal wastewater treatment plant, mixed with granular pine bark. The isolate was purified by subculturing on plate count agar (PCA; Merck) and maintained on brain heart infusion (BHI) agar. Cultures were incubated at 30 °C and cells were stored at −80 °C in nutrient broth with 15 % (v/v) glycerol for preservation. The colony and cell morphology, Gram-staining, cytochrome *c* oxidase and catalase tests, production of endospores and motility were analysed based on the methodologies of Murray *et al.* (1994) and Smibert & Krieg (1994). Unless otherwise stated, all biochemical and physiological tests were performed as described before (Vaz-Moreira *et al.*, 2007a). Biochemical and nutritional tests were performed using the API 20E, API 20NE and API 50CH galleries (bioMérieux) following the manufacturer's instructions. The API 50CH gallery was assayed with the medium recommended to test acid production (50 CHB/E; bioMérieux) and with enriched mineral medium B

(Vaz-Moreira *et al.*, 2007b) for single carbon source assimilation. Antibiotic susceptibility was assayed as described previously (Ferreira da Silva *et al.*, 2006).

Determination of the G+C content in genomic DNA and respiratory quinones were performed as described previously (Vaz-Moreira *et al.*, 2007a) using the methods of Mesbah *et al.* (1989) and Tindall (1989), respectively. The cellular fatty acid composition was analysed as described by Kämpfer & Kroppenstedt (1996). The major fatty acid methyl ester (FAME) components of strain SC-083^T were also analysed and identified by GC-MS, as described previously (Manaia & Moore, 2002). Purified peptidoglycan preparations were obtained after disruption of cells by shaking with glass beads and subsequent trypsin digestion, according to the method of Schleifer & Seidl (1985). The amino acid composition of the peptidoglycan hydrolysate (4 M HCl, 16 h, 100 °C) was determined by one-dimensional TLC on cellulose plates (Merck) by using the solvent system of Rhuland *et al.* (1955) and by GC and GC-MS of amino acids (Schumann *et al.*, 1997) after derivatization according to MacKenzie (1987). The acetyl or glycolyl type of the peptidoglycan was determined by the method of Uchida *et al.* (1999).

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 the GenBank database using the FASTA package from EMBL-EBI. Phylogenetic analysis was conducted using MEGA software version 3.1 (Kumar *et al.*, 2004). Sequence relatedness was estimated based on the model of Jukes & Cantor (1969) and the phylogenetic tree was created using the neighbour-joining method. Other methods, namely, maximum-parsimony, minimum evolution and unweighted pair-group method with arithmetic averages, were used to assess the tree stability. A total of 1340 nucleotide positions in each 16S rRNA gene were included in the analysis. Non-homologous and ambiguous nucleotide positions were excluded from the calculations.

Isolate SC-083^T presented good growth on BHI agar, forming white, opaque and convex colonies with 1–2 mm diameter after 48 h incubation at 30 °C. Slower and poorer growth was observed on other media such as PCA or Luria–Bertani agar (Carlton & Brown, 1981). This strain was able to grow on *Bacillus cereus* medium, turning it from pink to yellow, and was also able to grow in the presence of 0.001 % lysozyme. The isolate was able to produce acid from and to assimilate several sugars. The physiological and biochemical characteristics examined for SC-083^T are indicated in the species description. Other phenotypic tests included in the procedures described above and not referred to under the taxon description were negative for this isolate.

The fatty acid composition of strain SC-083^T, as well as that of type strains of closely related species, is presented in Table 1. The major respiratory quinones of this organism were menaquinones MK-11 and MK-12, with MK-10 as a minor component (56, 32 and 12 %, respectively). The muramic acid residues of the peptidoglycan were of the acetyl type and the amino acids ornithine, 2,4-diaminobutyric acid, glycine, alanine and glutamic acid were found in a molar ratio of 0.8 : 0.4 : 1.0 : 0.5 : 1.0. The DNA base composition determined for strain SC-083^T was 67.6 ± 0.2 mol% G+C.

The 16S rRNA gene sequence analysis suggested that strain SC-083^T forms a distinct lineage within the family *Microbacteriaceae*, with the species *Leifsonia xyli* (96 % similarity to the type strain), *Leifsonia shinshuensis* (96 %), *Leifsonia naganoensis* (95 %), *Leifsonia aquatica* (95 %), *Agromyces ramosus* (95 %) and *Curtobacterium citreum* (95 %) representing the closest phylogenetic neighbours (Fig. 1). The phylogenetic analysis, supported by the chemotaxonomic characterization, suggested that strain SC-083^T represents a new genus. The fatty acid cyclohexyl-$C_{17:0}$ was the major fatty acid in this organism and was not detected in its closest neighbours (Table 1), constituting a distinctive feature. Although unusual among the *Microbacteriaceae*, cyclohexyl-$C_{17:0}$ was reported before in three distinct species of *Curtobacterium*, *Curtobacterium pusillum*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* and *Curtobacterium ammoniigenes* (Aizawa *et al.*, 2007) and in *Leifsonia ginsengi* (Qiu *et al.*, 2007). Comparative analysis of the 16S rRNA gene sequences indicated a similarity of 94 % between strain SC-083^T and the type strains of these species, excluding those organisms from the group of the closest neighbours of the isolate under study. This observation was confirmed by different cell wall and menaquinone compositions that distinguished strain SC-083^T not only from other members of the *Microbacteriaceae* that produce cyclohexyl fatty acids, but also from its closest phylogenetic neighbours (Table 2). The combination of the diamino acids ornithine and 2,4-diaminobutyric acid in the peptidoglycan has been reported hitherto only for species of the genus *Agreia* (Evtushenko *et al.*, 2001; Schumann *et al.*, 2003), being a distinctive feature between strain SC-083^T and the genera *Leifsonia*, *Agromyces* and *Curtobacterium*. In these three genera, the diamino acids of the peptidoglycan are 2,4-diaminobutyric acid in *Leifsonia* and *Agromyces* and ornithine in *Curtobacterium*. The menaquinone composition also distinguished strain SC-083^T from the closest members of the genera *Agromyces* and *Curtobacterium*, with MK-12 and MK-9, respectively. Although most of the species in the genus *Leifsonia* possess mainly MK-11, some species, such as *L. shinshuensis*, may also have high percentages of MK-12, as was observed in strain SC-083^T (Suzuki *et al.*, 1999). Nevertheless, a 16S rRNA gene sequence similarity of 96 %, supported by a different cell wall and fatty acid composition, positions the two organisms in separate genera.

In contrast to the chemotaxonomic characteristics that allow a clear differentiation between the genera, most of the physiological and biochemical characteristics examined were observed to be variable among the different species of

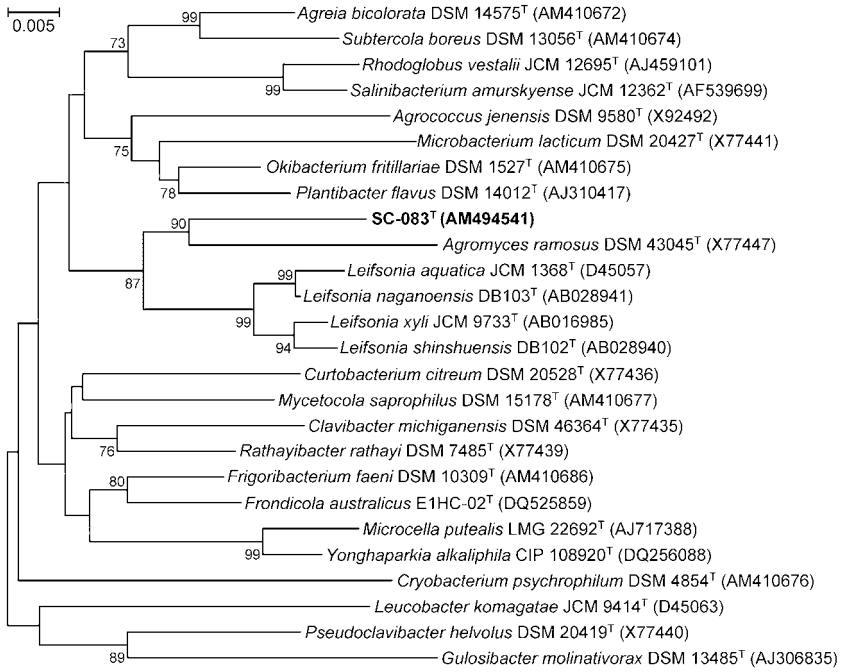

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain SC-083^T and its closest relatives of the genus *Leifsonia* and the type strains of the type species of the family *Microbacteriaceae*. Bootstrap values were generated from 1000 resamplings, only values greater than 60 % are shown. Bar, 1 substitution per 200 nucleotide positions.

Leifsonia, *Agromyces* and *Curtobacterium* and, hence, are considered of limited value to discriminate the proposed new genus from its relatives. The data presented herein are consistent with the proposal of a new genus and species, named *Humibacter albus* sp. nov., represented by type strain SC-083^T.

Description of *Humibacter* gen. nov.

Humibacter (Hu.mi.bac′ter. L. masc. n. *humus* earth, soil and, in earth sciences or agriculture, humus; N.L. masc. n. *bacter* rod; N.L. masc. n. *Humibacter* rod living in humus).

Table 1. Cellular fatty acid composition of strain SC-083^T and of related type strains

1, SC-083^T; 2, *Leifsonia aquatica* DSM 20146^T; 3, *Leifsonia naganoensis* DSM 15166^T; 4, *Leifsonia shinshuensis* DSM 15165^T; 5, *Curtobacterium citreum* DSM 20528^T; 6, *Agromyces ramosus* DSM 43045^T.

Fatty acid methyl ester	1	2	3	4	5	6
iso-$C_{14:0}$	0.3	0.6	0.6	0.4	1.1	0.6
iso-$C_{15:0}$	1.4	2.4	1.5	1.1	5.2	6.5
anteiso-$C_{15:0}$	11.0	39.9	42.8	40.9	52.5	41.7
iso-$C_{16:0}$	13.6	20.9	19.7	19.5	11.0	13.4
$C_{16:0}$	2.0	2.3	3.5	1.0	0.8	1.5
iso-$C_{17:0}$	1.2	1.0	0.6	0.6	1.2	2.5
anteiso-$C_{17:0}$	29.8	31.6	30.4	36.5	26.5	33.8
Cyclohexyl-$C_{17:0}$	39.4	–	–	–	–	–
Others*	1.3	1.3	0.9	–	1.7	–

*Others include $C_{14:0}$, anteiso-$C_{15:1}$, $C_{15:0}$, $C_{16:1}$, anteiso-$C_{17:1}$ and $C_{17:0}$.

Cells are Gram-positive, non-spore-forming, motile, short rods. Mesophilic. Strictly aerobic. Sugars are assimilated as carbon sources. The major respiratory quinones are MK-11 and MK-12, while MK-10 is a minor component. The predominant fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. The peptidoglycan contains ornithine in combination with 2,4-diaminobutyric acid. The muramic acid is acetylated. The DNA G+C content is approximately 68 mol%. Phylogenetically the genus belongs to the family *Microbacteriaceae*. The type species is *Humibacter albus*.

Description of *Humibacter albus* sp. nov.

Humibacter albus (al′bus. L. masc. adj. *albus* white).

Colonies are white, opaque and circular (1–2 mm diameter) on BHI agar. Cells are short rods (1.3 ± 0.22 μm long and 0.59 ± 0.08 μm wide). Coryneform V-shaped cells are formed during the early stages of growth. Growth occurs between 22 and 36 °C, between pH 5.5 and pH 8.0 and in the presence of 3 % NaCl, with optimum growth below 30 °C, pH around 7 and less than 1 % NaCl. Growth does not occur at 15 or 40 °C, at pH 9 and or in the presence of 5 % NaCl. Nitrate is reduced to nitrite, but does not support anaerobic growth. Catalase, β-galactosidase, urease and Voges–Proskauer tests are positive. Cytochrome *c* oxidase test is negative. Aesculin is hydrolysed. Assimilation and acid production occur in the presence of the following carbon sources: *N*-acetylglucosamine, amygdalin, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, glucose, D-lactose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-xylopyranoside, L-rhamnose, sucrose, salicin,

Table 2. Relevant chemotaxonomic characteristics of strain SC-083^T and related genera

Data for *Leifsonia*, *Agromyces* and *Curtobacterium* were obtained from Evtushenko *et al.*, 2000, Casida, 1984 and Jung *et al.*, 2007 and Komagata & Suzuki, 1984 and Behrendt *et al.*, 2002, respectively. S, Saturated; A, anteiso; I, iso; CH, cyclohexyl fatty acids; Orn, ornithine; DAB, 2,4-diaminobutyric acid.

	SC-083^T	*Leifsonia*	*Agromyces*	*Curtobacterium*
Major quinones	MK-11, MK-12	MK-11*†	MK-12*‡	MK-9
Cell wall diamino acid	Orn, DAB	DAB	DAB	Orn
Fatty acid types	S, A, I, CH	S, A, I, CH§	S, A, I	S, A, I, CH§
DNA G+C content (mol%)	68	66–73	65–73	68–75

*Other quinones that may be also present, representing one third or more of the total.
†MK-12 is also observed in *L. xyli* subsp. *cynodontis* and *L. shinshuensis*, MK-10 in *L. aquatica* and *L. naganoensis* (Suzuki *et al.*, 1999).
‡MK-13 in *A. subbeticus*, *A. neolithicus* and *A. humatus* (Jurado *et al.*, 2005a, b, c); MK-11 in *A. rhizospherae*, *A. salentinus* and *A. allii* (Takeuchi & Hatano, 2001; Jurado *et al.*, 2005a; Jung *et al.*, 2007).
§CH found only in some species, see text for details.

trehalose and D-xylose. Additionally, inositol, turanose and D-arabitol are assimilated and acid is produced from D-arabinose and D-ribose. Growth occurs in the presence of ceftazidime (30 μg) but not in the presence of amoxicillin (25 μg), gentamicin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), SXT (sulfamethoxazole/trimethoprim, 23.75/1.25 μg), sulfamethoxazole (25 μg), cephalothin (30 μg), streptomycin (10 μg), ticarcillin (75 μg), meropenem (10 μg) or colistin sulfate (50 μg). Cyclohexyl-$C_{17:0}$ is the major fatty acid. DNA G+C content of the type strain is 67.6±0.2 mol%. MK-11 (56 %) and MK-12 (32 %) are the major respiratory quinones and MK-10 (12 %) is a minority quinone.

The type strain is SC-083^T (=DSM 18994^T =LMG 23996^T =CCUG 54538^T), isolated from sewage sludge compost.

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