Microbacterium luticocti sp. nov., isolated from sewage sludge compost

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Strain SC-087B^T, isolated from sewage sludge compost during a study of bacterial diversity in composts, was characterized. The isolate was a Gram-positive, short rod that was motile, catalase- and oxidase-negative and able to grow at 27–45 °C, pH 5.5–9.7 and in up to 10 % NaCl. The peptidoglycan was of the B2β type, containing the characteristic amino acids ornithine, homoserine and hydroxyglutamic acid. The muramic acid residues of the peptidoglycan were partially glycolylated. The major cell-wall sugar was mannose; traces of xylose were also detected. The predominant fatty acids, comprising more than 70 % of the total, were anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$, the major respiratory quinone was menaquinone-12 (MK-12) and the G+C content of the genomic DNA was 72 mol%. Based on analysis of the 16S rRNA gene sequence, the closest phylogenetic neighbours of strain SC-087B^T were members of the family *Microbacteriaceae*, showing sequence similarity values of around 96 % with members of the species *Microbacterium barkeri* (96.0 %), *Microbacterium gubbeenense* (95.6 %) and *Microbacterium indicum* (95.7 %). The chemotaxonomic and phenotypic traits analysed supported the inclusion of this strain within the genus *Microbacterium* and the proposal of a novel species. The name *Microbacterium luticocti* sp. nov. is proposed and the type strain is SC-087B^T (=DSM 19459^T=CCUG 54537^T).

Composting is the biological decomposition of organic matter under aerobic conditions to produce a humus-like product that can be used for gardening or as a soil corrective. During this process, temperatures around 60 °C are reached, imposing stressful thermal conditions on the mesophilic microbiota (Epstein, 1997). In spite of this, mesophilic bacteria belonging to different phyla have been isolated from thermal composts (Tiago *et al.*, 2004; Vaz-Moreira *et al.*, 2008).

This paper describes a bacterial strain, designated SC-087B^T, isolated from municipal sewage sludge compost (Vaz-Moreira *et al.*, 2008). This compost was produced in a windrow digester from anaerobically digested sludge of a municipal wastewater treatment plant mixed with granular pine bark. 16S rRNA gene sequence analysis revealed that strain SC-087B^T was affiliated to the family *Microbacteriaceae*, with members of the genus *Microbacterium* as the closest neighbours (Takeuchi & Hatano, 1998a). This genus currently comprises more than 50 recognized species (Euzéby, 1997), including isolates from a broad range of origins, namely soil, air, water, dairy products, plant galls, insects, clinical samples and even culture contaminants (Takeuchi & Hatano, 1998b; Matsuyama *et al.*, 1999; Behrendt *et al.*, 2001; Schippers *et al.*, 2005; Richert *et al.*, 2007).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SC-087B^T is AM747814.

The isolate was purified by subculturing on plate count agar (PCA) and maintained on brain heart infusion (BHI) agar. Cultures were incubated at 30 °C and cells were stored at −80 °C in nutritive broth with 15 % (v/v) glycerol for preservation. Colony and cell morphologies, 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

previously (Vaz-Moreira *et al.*, 2007). Biochemical and nutritional tests were performed using the API 20E, API 20NE and API 50CH galleries (bioMérieux) according to the manufacturer's instructions. The API 50CH gallery was assayed with the medium recommended to test acid production (50 CHB/E; bioMérieux) and with AUX medium (bioMérieux) to test assimilation of sole carbon sources. Antibiotic susceptibility was assayed as described previously (Ferreira da Silva *et al.*, 2006).

The genomic DNA G+C content (mol%) and respiratory quinones were analysed as described by Vaz-Moreira *et al.* (2007) using the methods of Mesbah *et al.* (1989) and Tindall (1989), respectively. Fatty acid methyl esters were analysed on 24 h cultures on Columbia II agar base (BBL 4397596) with 5 % horse blood at 37 °C, according to the prevailing MIDI Sherlock MIS procedures (further outlined at http://www.ccug.se/pages/cfanew.pdf). 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 of amino acids (Schumann *et al.*, 1997) after derivatization according to MacKenzie (1987). The sugar composition of the purified cell wall was analysed by TLC on cellulose plates employing the method of Staneck & Roberts (1974). Glycolyl residues were detected in the peptidoglycan by using the method described by Uchida *et al.* (1999).

The nucleic acid 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/EMBL/DDBJ database using the FASTA package from EMBL-EBI. Phylogenetic analyses were 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 (Fig. 1). Additionally, the maximum-parsimony method was used to confirm tree stability. A total of 1285 nt positions in each 16S rRNA gene sequence was included in the analysis. Non-homologous and ambiguous nucleotide positions were excluded from the calculations.

Strain SC-087B^T formed convex, white, opaque colonies of 1–2 mm diameter after 48 h incubation at 30 °C on BHI

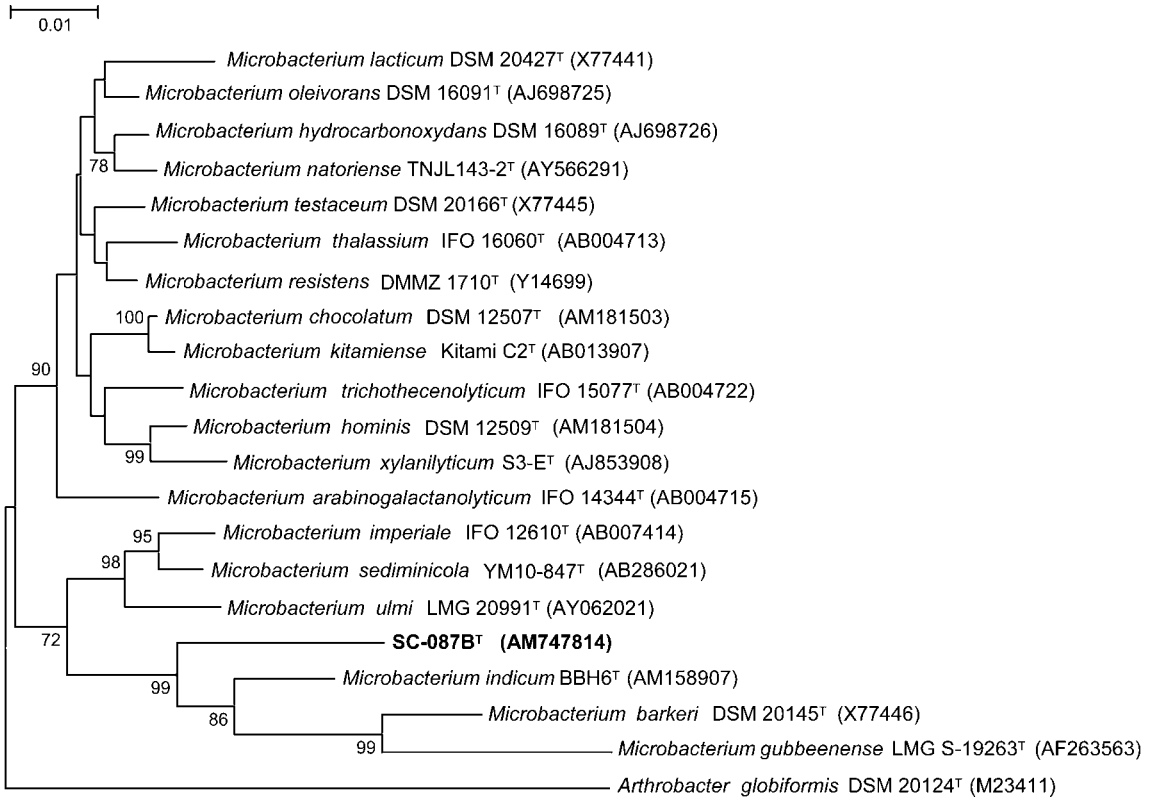

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the nearest neighbours of strain SC-087B^T. A total of 1285 nt positions in each 16S rRNA gene sequence was included in this analysis. *Arthrobacter globiformis* DSM 20124^T (M23411) was used as outgroup. Bootstrap values were generated from 1000 resamplings; only values greater than 70 % are shown. Bar, 1 substitution per 100 nt positions.

agar. Slower and poorer growth was observed on other nutritional media, e.g. PCA and Luria–Bertani agar. Cell viability was weakened or lost after periods of culture transfer longer than a week. The results of the phenetic characterization of strain SC-087B^T are summarized in Table 1. The peptidoglycan contained the amino acids ornithine, homoserine, glycine, alanine and glutamic acid. The molar ratio of glycine to glutamic acid was 1.7 : 1.0 and the glutamic acid was partially hydroxylated. Analytical data suggested the presence of peptidoglycan type B2β in strain SC-087B^T. The muramic acid residues of the peptidoglycan were partially glycolylated. Mannose and traces of xylose were detected as cell-wall sugars. The major fatty acid methyl esters of this organism were anteiso-$C_{17:0}$ (43.6 %) and anteiso-$C_{15:0}$ (32.9 %). Other minor components were iso-$C_{15:0}$ (9.5 %), iso-$C_{16:0}$ (9.2 %) and iso-$C_{17:0}$ (3.0 %). The DNA G+C content determined for strain SC-087B^T was 72 $\pm$ 0.3 mol%. Menaquinone MK-12 was the major respiratory quinone, with MK-11 and MK-10 as minor components (76, 23 and 1 %, respectively).

The closest neighbours of strain SC-087B^T based on analysis of the 16S rRNA gene sequence were *Microbacterium barkeri* (96.0 % sequence similarity), *Microbacterium gubbeenense* (95.6 % similarity) and *Microbacterium indicum* (95.7 % similarity). Important features described for the genus *Microbacterium* include: the predominance of iso- and anteiso-branched fatty acids; the presence of alanine, D-glutamic acid and either L-lysine, L-ornithine or L-homoserine in the peptidoglycan with an interpeptide bridge containing lysine or D-ornithine; muramic acid in the *N*-glycolyl form; the occurrence of MK-11 and MK-12 as major menaquinones; and a genomic DNA G+C content of 66–72 mol% (Takeuchi & Hatano, 1998a). Thus, on the basis of its chemotaxonomic characteristics, fatty acid and peptidoglycan composition, respiratory menaquinones and genomic DNA G+C content, strain SC-087B^T can be considered to be a member of the genus *Microbacterium*. However, 16S rRNA gene sequence similarity values with members of this genus, as well as the existence of distinctive characteristics (Table 1), justify the proposal of a novel species within the genus *Microbacterium* for which the name *Microbacterium luticocti* sp. nov. is proposed.

Table 1. Characteristics of strain SC-087B^T and the type strains of the related species *M. barkeri*, *M. gubbeenense* and *M. indicum*

Species/strains: 1, SC-087B^T; 2, *M. barkeri* (data from Komagata & Suzuki, 1984; Takeuchi & Hatano, 1998b; Brennan *et al.*, 2001); 3, *M. gubbeenense* (Brennan *et al.*, 2001; Shivaji *et al.*, 2007); 4, *M. indicum* (Shivaji *et al.*, 2007). +, Positive; −, negative; W, weak reaction; NA, no available data.

Characteristic	1	2	3	4
Catalase	−	+	+	−
Motility	+	+	+	NA
H_2S production	−	+	−	NA
Growth at/in:				
40 °C	+	+	−	−
8 % NaCl	+	−	+	−
Hydrolysis of:				
Starch	W	+	−	−
Gelatin	+	+	−	NA
Urease activity	+	−	−	−
Utilization of:				
L-Arabinose	−	+	+	+
Citrate	−	+	+	−
Malate	−	+	+	NA
Acid from:				
Arabinose	−	+	+	+
D-Galactose	W	−	+	−
D-Glucose	−	−	+	+
L-Rhamnose	+	+	−	−
Major fatty acids*	ai-C15, ai-C17	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16, C16
Cell wall diamino acid	Orn	Orn	Lys	Orn
Predominant menaquinones	12 and 11	11 and 12	11 and 12	11 and 12
DNA G+C content (mol%)†	72	68 (T_m)	72 (T_m)	66 (T_m)
Source	Sewage sludge compost	Raw domestic sewage	Smear-ripened cheese	Deep-sea sediment

*ai, anteiso; i, iso.

†T_m, Determined using the thermal denaturation method.

Description of *Microbacterium luticocti* sp. nov.

Microbacterium luticocti (L. neut. n. *lutum* mud, sludge; L. part. adj. *coctus -a -um* digested; N.L. gen. n. *luticocti* of digested sludge).

Colonies are white, opaque and circular (1–2 mm diameter) on BHI agar. Cells are Gram-positive, short rods (1.6 ± 0.3 μm long and 0.5 ± 0.1 μm wide) that are non-spore-forming and motile. Catalase- and oxidase-negative. Growth occurs between 27 and 45 °C, between pH 5.5 and 9.7 and in the presence of up to 10 % NaCl, with optimum growth around 36 °C, 1–3 % NaCl and pH 8. Growth does not occur at 25 °C or 47 °C, at pH 5 or pH 10, or in 12 % NaCl. Nitrate is reduced to nitrite, but does not support anaerobic growth. Starch, gelatin and aesculin are hydrolysed. Urease and β-galactosidase are produced. Acid is produced from D-arabitol, D-fructose, D-mannitol, L-rhamnose, sucrose and trehalose and produced weakly from cellobiose, L-fucose, D-galactose, maltose, D-mannose, potassium 5-ketogluconate and turanose. The following sole carbon sources are assimilated: *N*-acetylglucosamine, amygdalin, D-arabinose, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, D-lactose, maltose, D-mannitol, D-mannose, potassium gluconate, L-rhamnose, D-ribose, salicin and trehalose. Poor growth is observed when glucose is the single carbon source. Growth occurs in the presence of ciprofloxacin (5 μg), meropenem (10 μg), ceftazidime (30 μg), colistin sulfate (50 μg) and sulfamethoxazole (25 μg). Unable to assimilate L-arabinose, caprate, adipate, malate, citrate, phenylacetate, glycerol, erythritol, L-arabinose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, arbutin, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium 2-ketogluconate or potassium 5-ketogluconate. Does not produce acid from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-glucose, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, D-lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-arabitol, potassium gluconate or potassium 2-ketogluconate. Cannot ferment or oxidize (API 20E) D-glucose, D-mannitol, inositol, D-sorbitol, sucrose, melibiose, amygdalin or L-arabinose. Negative for Tweenase, β-galactosidase, arginine dihydrolase, lysine and ornithine decarboxylases, citrate utilization, and indole and acetoin production. Unable to grow in the presence of amoxicillin (25 μg), gentamicin (10 μg), tetracycline (30 μg), SXT (sulfamethoxazole/trimethoprim, 23.5/1.25 μg), cephalothin (30 μg), streptomycin (10 μg) or ticarcillin (75 μg). The fatty acids anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$ comprise more than 70 % of the total. The peptidoglycan is of the B2β type and contains glycolyl residues. Mannose is the cell-wall sugar. MK-12 is the major respiratory quinone.

The type strain is SC-087B^T (=DSM 19459^T=CCUG 54537^T), isolated from sewage sludge compost. The genomic DNA G+C content of the type strain is 72 ± 0.3 mol%.

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