Corynebacterium hansenii sp. nov., an α -glucosidase-negative bacterium related to *Corynebacterium xerosis*

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A novel strain, C-138^T, belonging to the genus *Corynebacterium* was isolated from a severe thigh liposarcoma infection and its differentiation from *Corynebacterium xerosis* and *Corynebacterium freneyi* is described. Analysis of 16S rRNA gene sequences, *rpoB* sequences and the PCR profile of the 16S–23S spacer regions was not conclusive enough to differentiate strain C-138^T from *C. xerosis* and *C. freneyi*. However, according to DNA–DNA hybridization data, strain C-138^T constitutes a member of a distinct novel species. It can be differentiated from strains of *C. xerosis* and *C. freneyi* by colony morphology, the absence of α -glucosidase and some biochemical characteristics such as glucose fermentation at 42 °C and carbon assimilation substrates. The name *Corynebacterium hansenii* sp. nov. is proposed for this novel species; the type strain is C-138^T (= CIP 108444^T = CCUG 53252^T).

Corynebacterium xerosis is rarely encountered in clinical samples (Riegel *et al.*, 1996; Funke *et al.*, 1996, 1997b; Wauters *et al.*, 1998). Consequently, the taxon *C. xerosis* has been excluded from the API (RAPID) coryne database 2.0 (Funke *et al.*, 1997a). In a recent publication (Vela *et al.*, 2006), eight isolates of *C. xerosis* were identified from animal clinical specimens. The situation became more confused with the description of *Corynebacterium freneyi* by Renaud *et al.* (2001) as, based on phylogenetic analyses, this species was considered to be closely related to *C. xerosis* and *Corynebacterium amycolatum*. Among the biochemical characteristics that differentiate these three species, α -glucosidase is known to be positive for *C. xerosis* ATCC 373^T and the reference strain ATCC 7711 (but not strain K132, isolated by Wauters *et al.*, 1998), negative for *C. amycolatum*, with some exceptions, and positive for *C. freneyi* (Renaud *et al.*, 2001; Auzias *et al.*, 2003). In this report, a novel species isolated from a human source that is α -glucosidase-negative is described.

†Died in 2004.

Abbreviation: ITS, intergenic spacer.

The GenBank/EMBL/DDBJ accession number of the partial 16S rRNA gene sequence of strain C-138^T is AY684044.

A 77-year-old patient with liposarcoma evolving over a 3 year period was recruited. Surgery was carried out to clean the swelling. The collected pus was cultured aerobically on sheep blood agar in an atmosphere with 10 % CO₂. Strain C-138^T was isolated in mixed culture with a few colonies of a methicillin-susceptible *Staphylococcus aureus* and some colonies of *Corynebacterium pseudodiphtheriticum*. Antibiotic susceptibility was determined using Mueller–Hinton medium supplemented with 5 % (v/v) sheep blood. Biochemical characteristics were determined using the API Coryne strip (bioMérieux). Other identification tests (glucose fermentation at 42 °C and growth at 20 °C) were performed according to Wauters *et al.* (1998). Carbon substrate assimilation tests were determined with the API 50CH system (bioMérieux) (Renaud *et al.*, 1998). Using the protocol described previously by Auzias *et al.* (2003), the partial sequence (952 bp) of the 16S rRNA gene was aligned and compared with all eubacterial 16S rRNA gene sequences available in the GenBank and EMBL databases. The partial *rpoB* gene sequence was amplified with primers C2700F and C3130R according to Khamis *et al.* (2004, 2005), except that the hybridization temperature was 51 °C instead of 57 °C.

Multiple-sequence alignments and similarities (%) of the *rpoB* and 16S rRNA genes between the various species were

Fig. 1. Dendrogram obtained by analysis of 16S rRNA gene sequences (tree method, fast minimum evolution). GenBank numbers are given in parentheses.

obtained with the CLUSTAL W program available from the EMBL-EBI web server (<http://www.ebi.ac.uk/clustalw/>). PCR analysis of the 16S–23S intergenic spacer region (ITS-PCR) was performed according to the method of Aubel *et al.* (1997). *CfoI* was used to digest the amplified products. *C. xerosis* ATCC 373^T, *C. amycolatum* CIP 103452^T and *C. freneyi* CIP 106767^T were also tested for profile comparison purposes. DNA was extracted and purified as described previously (Riegel *et al.*, 1994). Hybridization between labelled DNA of isolate C-138^T and the fragmented DNA preparations of *C. freneyi* CIP 106767^T, *C. xerosis* ATCC 373^T and *C. amycolatum* CIP 103452^T was carried out at 60 °C for 16 h in 0.42 M NaCl using the S1 nuclease-trichloroacetic acid method (Riegel *et al.*, 1994).

Gram staining of strain C-138^T revealed that it was a Gram-positive, non-spore-forming coryneform bacterium. After 48 h incubation, colonies were yellow-pigmented, very small, dry and rough. The API Coryne code for strain C-138^T was 2000325. Strain C-138^T was positive for pyrazinamidase, catalase and acid production from glucose, ribose, maltose and sucrose. Fermentation of sugars proceeded slowly during incubation (48 h). Biochemical differentiation of *C. xerosis*, *C. amycolatum* and *C. freneyi* was very difficult. Among the biochemical characteristics that differentiate these three species, α -glucosidase is known to be

positive for *C. xerosis* (except for strain K132) and *C. freneyi* and negative for *C. amycolatum*, with some exceptions, and the present strain (C-138^T). Strain C-138^T was able to grow at 20 °C, but did not ferment glucose at 42 °C. Assimilation tests (API 50CH) were positive for D-ribose, D-galactose, D-glucose, D-fructose and D-mannose and negative for all other substrates. Strain C-138^T was susceptible to almost all tested antibiotics, including amoxicillin, penicillin, imipenem, gentamicin, kanamycin, erythromycin, pristinamycin, rifampicin, vancomycin and teicoplanin. It was resistant to fosfomycin, pefloxacin and tetracycline. 16S rRNA gene sequence analysis showed that strain C-138^T was closely related to strains of *C. xerosis* and also *C. freneyi* (similarities >98%) and *C. amycolatum* (similarity >97%). As the similarity was always higher than 97%, this analysis does not constitute a reliable tool for identification (Stackebrandt & Goebel, 1994). The dendrogram obtained from sequence

Fig. 2. Dendrogram obtained by analysis of partial *rpoB* gene sequences (tree method, neighbour-joining). GenBank numbers are given in parentheses. The support of each branch, as determined from 1000 bootstrap samples, is indicated by the value at each node. *C. diphtheriae* is the outgroup strain.

Fig. 3. PCR profiles of the 16S–23S spacer regions of *Corynebacterium* species. Lanes: 1, *C. freneyi* CIP 106767^T; 2, *C. xerosis* ATCC 373^T; 3, strain C-138^T; 4, *C. amycolatum* CIP 103452^T; M, DNA ladder (Invitrogen). Profiles 2 and 3 are identical. Profiles 1 and 4 differ from each other and from that of *C. xerosis*.

Table 1. Characteristics that differentiate *C. hansenii* from *C. xerosis* and *C. freneyi*

Taxa: 1, *C. hansenii* sp. nov. C-138^T; 2, *C. xerosis* (Data from Funke *et al.*, 1997b and Wauters *et al.*, 1998); 3, *C. freneyi* strains CIP 106767^T and CIP 106768. +, Positive; –, negative.

Characteristic	1	2	3
Colony description	Yellowish, small, dry with irregular edges	Yellowish, small, dry with irregular edges	White, small, dry with irregular edges
Alkaline phosphatase	–	+	+
α -Glucosidase	–	+*	+
Fermentation of glucose at 42 °C	–	–	+
Assimilated substrates:			
D-Maltose	–	+	+
D-Turanose	–	+	+

*One exception.

analysis of various *Corynebacterium* strains is shown in Fig. 1; strain C-138^T is not included in the group of *C. xerosis* strains and is not linked to *C. freneyi*. Partial *rpoB* sequencing showed that the computed identities between strain C-138^T were 95 % with *C. freneyi* CIP 106767^T, 95 % with *C. xerosis* ATCC 373^T and 85 % with *C. amycolatum* CIP 103452^T (Fig. 2). This 95 % similarity value is the cut-off value for differentiating *Corynebacterium* species (Khamis *et al.*, 2005). In this case, the species could not be differentiated using the *rpoB* sequencing results. ITS-PCR analysis (Fig. 3) showed that the digestion profiles of strain C-138^T (lane 2) and *C. xerosis* (lane 3) were identical. The profiles of *C. freneyi* (lane 1) and *C. amycolatum* (lane 4) differed from each other. In a previous paper (Renaud *et al.*, 2001), the PCR profiles of three *C. amycolatum* strains, three *C. freneyi* strains and two *C. xerosis* strains were analysed. Profiles were identical within each species and differed between the species. This method seems to identify strain C-138^T as a strain of *C. xerosis*. The DNA–DNA hybridization values between strain C-138^T and the *C. xerosis*, *C. freneyi* and *C. amycolatum* type strains were <40 %, <47 % and <15 %, respectively. From the data, it is apparent that strain C-138^T is genomically different from *C. xerosis*, *C. freneyi* and *C. amycolatum* and should be assigned as a representative of a novel species.

Description of *Corynebacterium hansenii* sp. nov.

Corynebacterium hansenii (han.sen'i.i. N.L. gen. n. *hansenii* of Hansen, to honour Willy Hansen, a contemporary Belgian microbiologist who died in 2002).

The description given below and in Table 1 is based on the results of studies of one strain. Cells are Gram-positive, non-spore-forming and non-motile. They are typical club-shaped rods. Colonies are yellow-pigmented, very small (0.5–1.0 mm diameter), dry and rough. Growth is not encouraged in a medium containing lipids. Catalase- and

pyrazinamidase-positive. Produces acids from glucose, ribose, maltose and sucrose. Does not reduce nitrates. Does not produce alkaline phosphatase, pyrrolidonyl arylamidase, β -glucuronidase, β -galactosidase, α -glucosidase or *N*-acetyl- β -glucosaminidase. Does not hydrolyse aesculin, gelatin or urea. D-Ribose, D-galactose, D-glucose and D-fructose are used as carbon substrates, but D-maltose and D-turanose are not. Glucose is not fermented at 42 °C and growth occurs at 20 °C. Susceptible to almost all tested antibiotics except for fosfomycin, pefloxacin and tetracycline.

The type strain, C-138^T (=CIP 108444^T=CCUG 53252^T), was isolated from human liposarcoma pus. The pathogenic role of *C. hansenii* is not known. As the present isolate was found together with *C. pseudodiphtheriticum* and *S. aureus*, no conclusions can be drawn about its pathogenicity.

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