Characterization of clinical isolates previously identified as *Oerskovia turbata*: proposal of *Cellulosimicrobium funkei* sp. nov. and emended description of the genus *Cellulosimicrobium*

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Taxonomic studies were performed on 13 clinical isolates (ten of which were epidemiologically related) that had been previously identified as *Oerskovia turbata*. Comparative phylogenetic analysis, based on 16S rRNA gene sequences, indicated that the isolates are closely related to *Cellulosimicrobium cellulans* with sequence similarity values ranging from 99.5 to 99.8%. Chemotaxonomic results (fatty acid profiles and menaquinones) supported the inclusion of these isolates in the genus *Cellulosimicrobium*. The DNA G + C content was 74.5 mol%. The results of DNA–DNA reassociation, whole-cell sugars (with galactose as the characteristic whole sugar) and phenotypic properties, including antimicrobial resistance, indicated that these isolates are representatives of a novel species of the genus *Cellulosimicrobium*. The name *Cellulosimicrobium funkei* sp. nov. is proposed for the novel strains, with strain W6122^T (= ATCC BAA-886^T = DSM 16025^T = CCUG 50705^T) as the type strain. The definition of this novel *Cellulosimicrobium* species will assist in the understanding of the epidemiology and clinical significance of these micro-organisms.

The genus *Cellulosimicrobium* was first proposed by Schumann *et al.* (2001). At present, the genus comprises one species, *Cellulosimicrobium cellulans* (Schumann *et al.*, 2001). *Cellulosimicrobium variable*, proposed by Bakalidou *et al.* (2002), has been re-evaluated, transferred from the genus and reclassified as *Isoptericola variabilis* (Stackebrandt *et al.*, 2004).

In this paper, we describe the morphological, physiological and biochemical characteristics, antimicrobial susceptibility, whole-cell composition, cellular fatty acid and menaquinone content, 16S rRNA gene sequences and DNA–DNA hybridization of 13 clinical isolates (ten of which were epidemiologically related). A comparison was made with the type strains of *Cellulosimicrobium cellulans*, *Cellulomonas fimi*, *Cellulomonas hominis*, *Sanguibacter suarezii* and *Oerskovia turbata*. On the basis of the results of this

polyphasic study, the clinical isolates represent a novel taxon within the genus *Cellulosimicrobium*.

Strain W6122^T was isolated from the blood of an endocarditis patient and identified in 1975 as *O. turbata* (Reller *et al.*, 1975; Sottnek *et al.*, 1977). Recently, the clonality of W6122^T and three additional blood isolates (strains W2622, W2728 and W2729) from the same patient, as well as six homograft valve isolates (strains W2732, W2734, W2736, W2739, W6120 and W6121), was confirmed (McNeil *et al.*, 2004). In addition, McNeil *et al.* (2004) found that strain W6122^T was more closely related to *Cellulosimicrobium cellulans* (99.8% 16S rRNA gene sequence similarity) than to the type strain of *O. turbata* (95.3%). Strain W4083, isolated from a patient with acute myelogenous leukaemia with a catheter-related bacteraemia, was also described as *O. turbata* (LeProwse *et al.*, 1989; McNeil *et al.*, 2004). The two remaining strains, W2796 and W6123, were formerly identified as *O. turbata*, based primarily on phenotypic characteristics (Sottnek *et al.*, 1977; McNeil *et al.*, 2004). No clinical histories were available for either of these strains, except that strain W2796 was isolated from lung and strain W6123 was isolated from an unknown human source.

All isolates were inoculated onto heart infusion agar with 5% rabbit blood (BBL Microbiology Systems) and incubated

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The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of *Cellulosimicrobium funkei* strains W6122^T, W6123, W2796 and W4083 are AY501364 and AY523787–AY523789, respectively.

at 25 and 35 °C for 2 days for morphological studies. Gram stain was used to study microscopic morphology. The isolates were examined for the presence of aerial and substrate hyphae at low power ($\times 10$ magnification) under a stereomicroscope. Phenotypic characterization was performed using previously described methods (McNeil *et al.*, 2004) and results are given in the species description. Antimicrobial susceptibilities were determined by a previously described broth microdilution method with cation-supplemented Mueller–Hinton broth (NCCLS, 1997). The antimicrobial agents tested were amikacin, amoxycillin–clavulanic acid, ampicillin, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, minocycline, trimethoprim–sulfamethoxazole and vancomycin. Plates were incubated at 25 or 35 °C for 48 h. Since the methods of testing and the break-points for resistance for the genus have not been standardized or approved by the NCCLS, the break-points used for resistance were those of NCCLS for organisms that grow aerobically (NCCLS, 1997). The break-point for resistance for clarithromycin was taken from the recently NCCLS-approved standard for the susceptibility testing of mycobacteria, nocardiae and other aerobic actinomycetes (NCCLS, 2003).

The genomic DNAs of the isolates were purified from lysed protoplasts as described previously (Lasker *et al.*, 1992). DNA labelling and DNA–DNA relatedness experiments were performed with strain W6122^T and strains W2796, W4083 and W6123 and the type strains of *Cellulosimicrobium cellulans* and *O. turbata* using the hydroxyapatite method described by Brenner *et al.* (1982). Strain W6122^T was related to strains W2796 (76 %), W4083 (78 %) and W6123 (79 %), with a divergence (ΔT_m) of 1.5, 2.0 and 1.5 %, respectively, but showed low DNA–DNA relatedness to *Cellulosimicrobium cellulans* ATCC 12830^T (47 %) and *O. turbata* ATCC 25835^T (3.0 %).

The DNA G+C content was determined spectrophotometrically by thermal denaturation as described previously (Mandel *et al.*, 1970). The DNA G+C content of strain W6122^T was 74.5 mol%. This value was consistent with the DNA G+C content of 74 mol% observed for the genus *Cellulosimicrobium* (Schumann *et al.*, 2001).

The 16S rRNA genes of strains W6122^T, W2796, W4083, W6123 and *Cellulosimicrobium cellulans* ATCC 12830^T were analysed as described by McNeil *et al.* (2004). A continuous stretch of 1444 nt of the 16S rRNA gene sequence was determined and used for comparative phylogenetic analysis. Related sequences were identified in a BLAST search against the GenBank database. Sequences were aligned and a distance matrix was created with CLUSTAL W. A phylogenetic tree was constructed in TREECON with the neighbour-joining method (Fig. 1) and bootstrapped with 1000 replications (McNeil *et al.*, 2004). The 16S rRNA gene sequence similarity values between strains W6122^T, W2796, W4083 and W6123 ranged from 99.8 to 100 %. The sequence similarity values between these strains and *Cellulosimicrobium cellulans* ATCC 12830^T ranged from 99.5 to 99.8 %.

Results of the chemotaxonomic analyses are given in the species description. The following analytical procedures were performed: diaminopimelic acid and monosaccharide whole-cell analysis (Berd, 1973), cellular fatty acid analysis (Weyant *et al.*, 1996) and quinone analysis (Moss & Guerrant, 1983). Whole-cell analyses of the organisms indicated that they contained neither the *meso*- nor the *L*-isomer of diaminopimelic acid. Galactose was the only characteristic whole cell-wall sugar found. The presence of only galactose is not consistent with the cell-wall sugars identified from *Cellulosimicrobium cellulans*; thus, this characteristic may be useful for species differentiation (Stackebrandt *et al.*, 2004).

The strains share a similar respiratory quinone profile that is characterized by large amounts of menaquinone-9 [MK-9(H₄)]. Predominant fatty acids are 12-methyltetradecanoic acid (ai-C_{15:0}), 14-methylpentadecanoic acid (i-C_{16:0}), hexadecanoic acid (C_{16:0}) and 13-methyltetradecanoic acid (i-C_{15:0}). The fatty acid profile of strain W6122^T was similar to that of *Cellulosimicrobium cellulans* ATCC 12830^T.

In contrast to the chemotaxonomic similarities between strains W6122^T and *Cellulosimicrobium cellulans*, the only other species of the genus *Cellulosimicrobium*, clear differences were seen in the results of phenotypic tests (Table 1).

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *Cellulosimicrobium funkei* sp. nov. strains W4083, W2796, W6123 and W6122^T and *Cellulosimicrobium cellulans* ATCC 12830^T. The tree was rooted by using *Cellulomonas fimi* as the outgroup. Bar, 2 % difference in nucleotide sequence.

Table 1. Phenotypic characteristics that differentiate strains W6122^T, W2796, W4083 and W6123 from related species and genera

Reference strains: 1, *Cellulosimicrobium cellulans* ATCC 12830^T; 2, *Cellulomonas hominis* ATCC 51964^T; 3, *Cellulomonas fimi* ATCC 484^T; 4, *Sanguibacter suarezii* ATCC 51766^T; 5, *Oerskovia turbata* ATCC 25835^T. All strains were positive for acid production from fructose, D-galactose, D-glucose, lactose, maltose, salicin, sucrose, D-trehalose and D-xylose. All strains were negative for acid production from adonitol, dulcitol, i-erythritol, *myo*-inositol and D-mannitol. All strains were negative for the hydrolysis of tyrosine. All strains grew at 25 °C, but not at 45 °C. All strains were susceptible to amoxycillin–clavulanic acid, ampicillin, ceftriaxone, clarithromycin, minocycline and vancomycin. All strains have a convex and entire-edged macroscopic morphology. In addition, *Sanguibacter suarezii* ATCC 51766^T has been described as having a ‘fried egg’ morphology. Data for the type strains of related species and genera were derived from the CDC’s Meningitis and Special Pathogens Branch database. For antibiotic resistance, the minimum inhibitory concentration (MIC) resistance break-points used are those of the NCCLS (1997, 2003). +, Positive; –, negative; (+), weakly positive; HIA, heart infusion agar.

Characteristic	<i>Cellulosimicrobium funkei</i> sp. nov.				1	2	3	4	5
	W6122 ^T	W2796	W4083	W6123					
Acid fermentation of:									
L-Arabinose	+	+	–	+	+	+	+	+	+
Cellobiose	+	+	+	+	(+)	+	+	+	+
Glycerol	+	+	–	+	–/+*	+	–	–	+
Inulin	–	–	–	–	+	–	–	–	–
Mannose	+	+	–	+	+	+	+	+	+
Melezitose	–	–	–	–	–	–	+	–	–
Melibiose	–	+	–	–	+	–	+	+	–
Raffinose	–	–	–	–	(+)	–	+	+	–
L-Rhamnose	–	–	–	–	–	+	+	+	–
D-Sorbitol	–	–	–	–	–	–	–	+	–
Growth at 35 °C	+	+	+	+	+	+	+	–	+
Pigment on HIA slants in 3 days	Yellow	Yellow	Yellow	Yellow	Yellow	Pale-yellow	Pale-yellow	Yellow	Yellow
Hydrolysis of:									
Adenine	+	+	+	+	+	–	–	–	–
Casein	+	+	+	+	+	–	–	–	–
Hypoxanthine	+	+	+	+	+	–	–	–	–
Xanthine	+	+	+	+	+	–	–	–	–
Substrate hyphae	+	+	+	+	+	–	–	–	+
Motility	+†	+	+	+	–	+	+	+	+
Urease production (Christensen urea agar slant)	+	+	(+)	(+)	+	+/-‡	–	–	–
Nitrate reduction	–	+	+	+	+	+	+	+	+
Gelatin liquefaction (14 days)	+	+	+	+	+	–/+‡	–	–	+
Resistance to:									
Amikacin (MIC ≥ 64 µg ml ⁻¹)	+	+	+	+	+	+	+	+	–
Ciprofloxacin (MIC ≥ 4 µg ml ⁻¹)	+	+	+	+	+	–	+	+	+
Imipenem (MIC ≥ 16 µg ml ⁻¹)	–	–	–	–	+	–	–	–	–
Trimethoprim–sulfamethoxazole (MIC ≥ 4–76 µg ml ⁻¹)	+	+	+	+	–	+	+	–	–

*Schumann *et al.* (2001) described acid production from glycerol.

†One to five polar or/or lateral flagella were seen on all motile isolates (Sottnek *et al.*, 1977).

‡Funke *et al.* (1995) described *Cellulomonas hominis* as urease-negative using Christensen broth and gelatin-positive using the film strip method.

Strains W6122^T, W2796, W4083 and W6123 lacked the ability to produce acid from inulin and raffinose and were motile, whereas *Cellulosimicrobium cellulans* ATCC 12830^T produced acid from inulin and raffinose and was non-motile. Table 1 lists additional phenotypic properties that distinguish strains W6122^T, W2796, W4083 and W6123 and *Cellulosimicrobium cellulans* ATCC 12830^T from related

species and genera. The high levels of DNA–DNA relatedness demonstrate that these isolates represent the same species and the low levels of sequence similarity to other genera support the distinctness of these isolates (Fig. 1).

Microscopic morphological studies showed that all isolates studied were Gram-positive, pleomorphic bacilli, lacking

spores and capsules. Under low-power stereomicroscopic examination, the isolates showed pale-yellow to yellow, small diameter (approx. 1 mm) colonies after incubation on heart infusion agar with 5 % (v/v) rabbit blood for 2 days at 35 °C. Substrate hyphae were seen. All colonies were smooth, convex and entire-edged. All isolates studied were motile, with lateral or polar flagella or both (Sottnek *et al.*, 1977).

Emended description of *Cellulosimicrobium* Schumann *et al.* 2001

Cellulosimicrobium (Cell.u.lo.si.mi.cro'bi.um. N.L. n. *cellulosa* cellulose; Gr. adj. *mikros* small; Gr. masc. n. *bios* life; N.L. neut. n. *Cellulosimicrobium* cellulose microbe).

The Neo Latin noun *Cellulosimicrobium* is in the neuter gender and not in the feminine gender as cited by Schumann *et al.*, 2001 [all Latin names which end in *-um* (nominative singular) are assigned neuter gender].

The description is as given by Schumann *et al.*, 2001, with the following modifications. Whole-cell sugars are galactose, rhamnose, glucose, fucose and mannose or galactose and ribose. Motile or non-motile. Nitrate reduction is positive or negative.

Description of *Cellulosimicrobium funkei* sp. nov.

Cellulosimicrobium funkei (fun'ke.i. N.L. gen. masc. n. *funkei* of Funke, to honour the contributions of Guido Funke, a distinguished microbiologist, who recognized the heterogeneity within the yellow-pigmented coryneform bacteria).

Cells are short (1 µm), thin, Gram-positive and non-sporulating rods. Motile by polar and/or lateral flagella (Sottnek *et al.*, 1977). Growth occurs at 35 and 45 °C, but not at 25 °C. Pale-yellow pigment. Substrate hyphae are present. Catalase-positive. Aesculin, adenine, casein, gelatin, hypoxanthine, urea and xanthine are decomposed. Nitrate is not reduced to nitrite (Sottnek *et al.*, 1977). Acid is produced from L-arabinose, cellobiose, fructose, D-galactose, D-glucose, glycerol, lactose, maltose, mannose, salicin, sucrose, D-trehalose and D-xylose. No acid production from adonitol, dulcitol, i-erythritol, myo-inositol, inulin, D-mannitol, melezitose, melibiose, raffinose, L-rhamnose or D-sorbitol. DNA G + C content is 74.5 mol%. The characteristic whole-cell sugar is galactose.

The type strain, W6122^T (=ATCC BAA-886^T=DSM 16025^T=CCUG 50705^T), was isolated from human blood.

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