NOTE

Reclassification of *Cellulomonas cellulans* (Stackebrandt and Kieddie 1986) as *Cellulosimicrobium cellulans* gen. nov., comb. nov.

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Phylogenetic analysis of 16S rDNA provides evidence that *Cellulomonas cellulans* branches outside the phylogenetic confines of the genus *Cellulomonas*. The distinct phylogenetic and chemotaxonomic status of *Cellulomonas cellulans* as a phylogenetic neighbour of the genus *Promicromonospora*, justifies the description of a new genus for which the name *Cellulosimicrobium* gen. nov. with the type species *Cellulosimicrobium cellulans* comb. nov. is proposed.

Keywords: *Cellulomonas*, *Cellulosimicrobium* gen. nov., *Cellulosimicrobium cellulans* comb. nov.

The genus *Cellulomonas* is a member of the suborder *Micrococcineae*, order *Actinomycetales*, class *Actinobacteria*. Most members show cellulolytic activity and one species, *Cellulomonas hominis* (Funke *et al.*, 1996) has been isolated from clinical material. Besides those species that have been members of the genus since its original description (Bergey *et al.*, 1923), i.e. *Cellulomonas flavigena*, *Cellulomonas biazotea*, *Cellulomonas cellasea*, *Cellulomonas gelida*, *Cellulomonas fimi* and *Cellulomonas uda*, a few species have been added more recently, i.e. *Cellulomonas cellulans* (basonym '*Nocardia cellulans*', embracing the misclassified species '*Cellulomonas cartae*', '*Brevibacterium lyticum*', '*Brevibacterium fermentans*' and '*Oerskovia xanthineolytica*'; Stackebrandt & Kandler, 1980), *Cellulomonas fermentans* (Bagnara *et al.*, 1985), *Cellulomonas hominis* (Funke *et al.*, 1996), *Cellulomonas persica* and *Cellulomonas iranensis* (Elberson *et al.*, 2000). In addition, *Cellulomonas (Oerskovia) turbata* was affiliated to the genus as a consequence of the union of *Cellulomonas* and *Oerskovia* (Stackebrandt *et al.*, 1982).

The taxonomic status of *Cellulomonas cellulans*

16S rDNA sequence analyses of members of the suborder *Micrococcineae* revealed the emergence of lineages, which, based upon the distribution of sig-

nature nucleotides, were described as families (Stackebrandt *et al.*, 1997). Among these are the families *Cellulomonadaceae*, embracing the genus *Cellulomonas*, and *Promicromonosporaceae*, containing the genus *Promicromonospora*. Since then, several new genera of this suborder have been described (Groth *et al.*, 1997a, b, 1999; Martin *et al.*, 1997) which has changed the phylogenetic position of a few taxa and stabilized other taxa. As a consequence a few new families have been described, i.e. *Bogoriellaceae*, *Dermacoccaceae*, *Rarobacteraceae* and *Sanguibacteraceae* (Stackebrandt & Schumann, 2000). Also, the inclusion into the phylogenetic analysis of several sequence entries deposited in databases of unclassified *Cellulomonas* strains (accession nos AB023360–AB023367) reinforced the finding that *Cellulomonas cellulans* grouped more distantly from the core of *Cellulomonas* than observed previously (Rainey *et al.*, 1995). Fig. 1 shows the clustering of the type strain of *Cellulomonas cellulans* next to *Promicromonospora citrea* and *Promicromonospora sukumoe*.

The topology of the 16S rDNA dendrogram, recovered by the two phylogenetic methods applied [distance matrix (DeSoete, 1983) and maximum-likelihood (Felsenstein, 1993)], suggests that *Cellulomonas cellulans* is not a member of the authentic genus *Cellulomonas* (Fig. 1). However, the branching point of *Cellulomonas cellulans* outside *Cellulomonas* proper should not be used as the sole criterion for reclassification purposes, in as much as this branching point is not supported by bootstrap values of high significance.

The GenBank accession number for the 16S rDNA sequence of *Promicromonospora sukumoe* is AJ272024.

Fig. 1. Phylogenetic relatedness among members of *Cellulosimicrobium*, *Cellulomonas* and some related genera of the suborder *Micrococccineae*, order *Actinomycetales*, class *Actinobacteria*, based upon 16S rDNA sequence comparison. The dendrogram was generated by distance analysis (DeSoete, 1983), following Kimura two-parameter corrections of similarity values. Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 100 bootstrapped trees (only values of 70% and above are shown). Regions of the tree in which the branching order of lineages depends upon the treeing algorithms used (distance matrix and maximum-likelihood) are indicated as hatched lines. Sequences of species of other actinobacterial suborders served as a root. The scale bar represents 2 nt substitutions per 100 nt.

Table 1. Diagnostic chemotaxonomic properties of type strains of *Cellulosimicrobium* and some *Cellulomonas* species

Orn, Ornithine; Asp, aspartic acid; Glu, glutamic acid; Lys, lysine; Ser, serine; Thr, threonine; Ala, alanine; Rha, rhamnose; Man, mannose; Rib, ribose; Gal, galactose; Tal, talose; Fuc, fucose; Glc, glucose; Glc-NH₂, glucosamine; ND, not determined. Abbreviations of fatty acids exemplified by ai-C_{17:0}, 14-methylhexadecanoic acid; i-C_{15:0}, 13-methyltetradecanoic acid; C_{16:0}, hexadecanoic acid.

Species	Diamino acid of peptidoglycan*	Interpeptide bridge*	Cell wall sugars†	Predominant cellular fatty acids‡	G + C content (mol%)†
<i>Cellulosimicrobium cellulans</i>	L-Lys	D-Ser ← D-Asp	Rha, Fuc, Gal	ai-C _{15:0} , i-C _{16:0} , C _{16:0} , i-C _{15:0}	74
<i>Cellulomonas flavigena</i>	L-Orn	D-Asp	Rha, Man, Rib	ai-C _{15:0} , C _{16:0} , C _{14:0}	72.7–74.8
<i>Cellulomonas biazotea</i>	L-Orn	D-Glu	Rha, Gal, Man, 6-desoxy-Tal	ai-C _{15:0} , i-C _{15:0} , C _{16:0}	71.5–75.6
<i>Cellulomonas cellasea</i>	L-Orn	D-Glu	Rha, Man, 6-desoxy-Tal	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}	75.0
<i>Cellulomonas fermentans</i>	L-Orn	ND	Glc, Rha, Rib	ai-C _{15:0} , C _{16:0} , C _{14:0}	75.8
<i>Cellulomonas fimi</i>	L-Orn	D-Glu	Rha, Fuc, Glc	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}	71.3–72.0
<i>Cellulomonas gelida</i>	L-Orn	D-Glu	Glc	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}	72.4–74.4
<i>Cellulomonas hominis</i>	L-Orn	D-Glu	ND	ai-C _{15:0} , C _{16:0} , C _{14:0} , C _{15:0}	76
<i>Cellulomonas uda</i>	L-Orn	D-Glu	Glc, Man	ai-C _{15:0} , C _{16:0} , C _{15:0}	72

* Data from Fiedler & Kandler (1973), Stackebrandt *et al.* (1978), Seidel *et al.* (1980), Bagnara *et al.* (1985), DSMZ Catalogue of Strains (1998) and this paper.

† Data from Bagnara *et al.* (1985) and Stackebrandt & Keddle (1986). G + C data for *Cellulosimicrobium cellulans* from this paper.

‡ Data from Funke *et al.* (1995) and this paper.

The rationale for a reclassification of this species as the nucleus for a new genus, adjacent to the genus *Promicromonospora*, is also based upon the presence of the peptidoglycan type A4α (Lys ← D-Ser ← D-Asp) in the type strain of *Cellulomonas cellulans* and other strains of this species (Stackebrandt *et al.*, 1978), and the absence of this peptidoglycan type in strains of authentic *Cellulomonas* species (Table 1) and *Promicromonospora* species. The agreement between the phylogenetically distinct position of an organism in the

16S rDNA dendrogram and the unique combination of chemotaxonomic properties (e.g. peptidoglycan, polar lipids, fatty acids, menaquinones) exhibited by this organism has been the basis for many recent new genus descriptions within the suborder *Micrococccineae* (Fig. 1).

The distinct phylogenetic and chemotaxonomic status of *Cellulomonas cellulans* justifies the description of a new genus for which the name *Cellulosimicrobium*

gen. nov. is proposed. The type species is *Cellulosimicrobium cellulans* comb. nov.

Description of *Cellulosimicrobium* gen. nov.

Cellulosimicrobium (Cel.lu.lo.si.mi.cro'bi.um. M.L. n. *cellulosa* cellulose; Gr. adj. *micro* small; Gr. masc. n. *bios* life; M.L. fem. n. *Cellulosimicrobium* cellulose microbe).

The following description is based on the description of the species *Nocardia cellulans* DSM 43879^T (this study; Stackebrandt *et al.*, 1980) *Cellulomonas cartae* (Stackebrandt & Kandler, 1980), considered a subjective synonym of *Cellulomonas cellulans* (Stackebrandt & Keddie, 1986), basonym *Nocardia cellulans*. The fatty acid composition has also been determined by Funke *et al.* (1995) for *Oerskovia xanthineolytica* CIP 81.28. Fatty acid composition, polar lipids and base composition of the DNA of strain DSM 43879^T followed described methods (Groth *et al.*, 1997a, b). In young cultures a primary mycelium is produced which fragments later into irregular, curved and club-shaped rods which may be arranged in V forms. After exhaustion of the medium, the rods are transformed into shorter rods or even spherical cells. Do not form endospores. Gram-positive, but cells are very readily decolorized. Not acid-fast. Non-motile. Chemo-organotrophic; metabolism aerobic and facultatively anaerobic. Acid produced from a wide variety of carbohydrates. Catalase-positive. Cellulolytic. Nitrate reduction positive. Peptidoglycan contains L-lysine; the interpeptide bridge consists of D-Ser ← D-Asp, type A4α. Major menaquinone is 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}). G + C content of the DNA is 74 mol% (T_m). Type species is *Cellulosimicrobium cellulans*.

Description of *Cellulosimicrobium cellulans* (Metcalf and Brown 1957) gen. nov., comb. nov.

Cellulosimicrobium cellulans (cel'lu.lans. N.L. part. adj. *cellulans* cell-making).

Basonym *Nocardia cellulans*. The following description is based on that of *Nocardia cellulans* DSM 43879^T (this study; Stackebrandt *et al.*, 1980; Stackebrandt & Keddie, 1986; Funke *et al.*, 1995), *Cellulomonas cartae* DSM 20106, '*Corynebacterium manihot*' DSM 20155, *Brevibacterium fermentans* CIP 6611 and *Brevibacterium lyticum* ATCC 15921 (Stackebrandt & Kandler, 1980; Stackebrandt *et al.*, 1982) and for *Oerskovia xanthineolytica* (Stackebrandt *et al.*, 1980; Funke *et al.*, 1995). A primary mycelium is produced which fragments later in the growth cycle. After exhaustion of the medium, the rods transform into shorter rods or even spherical cells. On peptone-yeast extract-glucose agar, colonies are circular, 0.9–5 mm in diameter, convex yellow-whitish and

glistening, edge entire. The following are used as sole carbon sources: glucose, mannose, maltose, sucrose, D-xylose, L-arabinose, D-ribose, m-inositol, glycerol, cellobiose, lactose, gluconate, mannitol, L-lactate, acetate, pyruvate, propionate, pentanoate, capronate, heptanoate, caprylate, glyoxylate, proline, asparagine, aspartate and histidine. Raffinose, DL-malate and D-lactate are not used as sole carbon sources. Xanthine is hydrolysed. Acid is produced from glucose, mannose, sucrose, D-xylose, L-arabinose, ribose, gluconate, glycerol, lactose, pyruvate and cellobiose. Starch is hydrolysed and gelatin slowly liquefied. Catalase is produced. Nitrite is produced from nitrate. Aerobic and facultatively anaerobic. Acetic acid is the main acidic intermediary product of aerobic glucose dissimilation. Resting cells ferment glucose anaerobically predominantly to CO₂, acetic acid and L-lactic acid. Ethanol and formic acid are minor end products. Peptidoglycan contains L-lysine; the interpeptide bridge consists of D-Ser ← D-Asp, type A4α. The major cell wall sugar is rhamnose, while fucose and galactose are minor components. Major menaquinone is MK-9(H₄); MK-9(H₂), MK-8(H₄) and MK-7(H₄) occur as minor components. 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 G + C content of the DNA is 74 mol% (T_m). Type strain is DSM 43879^T.

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