Reclassification of *Cellulosimicrobium variabile* Bakalidou *et al.* 2002 as *Isoptericola variabilis* gen. nov., comb. nov.

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As already depicted in the original publication, the type strain of the species *Cellulosimicrobium variabile* Bakalidou *et al.* 2002, DSM 10177^T, does not cluster unambiguously with the type species, *Cellulosimicrobium cellulans* DSM 43879^T, in phylogenetic analysis. Strain DSM 10177^T is moderately related to the recently described species *Xylanimonas cellulosilytica*, *Promicromonospora pachnodae* and *Xylanibacterium ulmi*, forming a lineage that branches between *C. cellulans* and members of the genus *Promicromonospora* in most dendrograms generated on the basis of different algorithms and reference strains. The type strains of the two *Cellulosimicrobium* species resemble each other in morphology, composition of fatty acids, DNA G + C content, phospholipids and the presence of lysine in position 3 of the peptide subunit of peptidoglycan. However, the two strains differ from each other in cell-wall sugars and in the amino acid composition of the A4 α -type peptidoglycan, which contains serine and aspartic acid in *C. cellulans*, whereas only aspartic acid is present in the interpeptide bridge of *C. variabile*. This type is also present in *Xylanimonas cellulosilytica* XIL07^T, but not in the neighbouring species *P. pachnodae* DSM 12657^T, which exhibits the L-Lys–L-Ser–D-Glu type. On the basis of distinct phylogenetic position and the amino acid composition of peptidoglycan, a novel genus and combination for *C. variabile*, *Isoptericola variabilis* gen. nov., comb. nov., is proposed.

The genus *Cellulosimicrobium* (Schumann *et al.*, 2001) is a member of the suborder *Micrococcineae*, order *Actinomycetales*, class *Actinobacteria* (Stackebrandt *et al.*, 1997) and is related to the recently described genus *Xylanimonas* (Rivas *et al.*, 2003), the species *Promicromonospora pachnodae* (Cazemier *et al.*, 2003) and, more distantly, to the genera *Cellulomonas*, *Terrabacter*, *Rarobacter* and relatives (Schumann *et al.*, 2001; Stackebrandt *et al.*, 2002). The genus *Cellulosimicrobium* contains two species at the time of writing, *Cellulosimicrobium cellulans* (Schumann *et al.*, 2001) and *Cellulosimicrobium variabile* (Bakalidou *et al.*, 2002). In contrast to their generic affiliation (96.6% 16S rRNA gene sequence similarity), the two species did not group together in 16S rRNA gene sequence analysis (GenBank accession numbers are indicated in Fig. 1), as already shown in the original description of *C. variabile* DSM 10177^T (Bakalidou *et al.*, 2002). This strain, together with *P. pachnodae* (Cazemier *et al.*, 2003), *Xylanimonas cellulosilytica* (Rivas *et al.*, 2003) and *Xylanibacterium ulmi* (Rivas *et al.*, 2004), which share 97.0–97.3% gene sequence similarity, branch within the *Promicromonospora* subline of descent (Fig. 1). These latter values are only slightly higher than those shared between *C. cellulans* and the type strains

of the four authentic *Promicromonospora* species (95.2–95.6%), demonstrating the intermediate position of *C. variabile*, *P. pachnodae*, *Xylanibacterium ulmi* and *Xylanimonas cellulosilytica* between *C. cellulans* (94.9–96.6%) and authentic *Promicromonospora* species (95.3–96.5%). By applying different treeing algorithms [such as neighbour-joining, maximum-likelihood (Felsenstein, 1993) and the additive treeing algorithm of DeSoete (1983)] to sequences of these organisms and relatives, the branching of *C. variabile* DSM 10177^T and *Xylanimonas cellulosilytica* with members of the genus *Promicromonospora* was confirmed in most cases. Small changes in branching points of less closely related organisms were observed when the number and selection of reference organisms were changed (data not shown).

The generic affiliation of *C. variabile* was based on the presence of the following characters: presence of peptidoglycan type A4 α (L-lysine as the diamino acid and aspartic acid in the interpeptide bridge) and galactose and glucose in the cell wall. The description of the peptide structure of *C. variabile* needs discussion. The murein, as published, contained L-lysine, aspartic acid, glutamic acid and alanine

Fig. 1. Phylogenetic relatedness among members of the genera *Cellulosimicrobium*, *Xylanimonas*, *Xylanibacterium*, *Promicromonospora*, *Cellulomonas* and *Oerskovia*, based on 16S rDNA sequence comparison. *Beutenbergia cavernae* DSM 12333^T (GenBank no. Y18378) and *Jonesia denitrificans* DSM 20603^T (GenBank no. X83811) served as a root. The dendrogram was generated by neighbour-joining analysis (Felsenstein, 1993). Numbers within the dendrogram indicate percentages of occurrence of the branching order in 100 bootstrapped trees (only values of 50% and above are shown). Bar, 5 nucleotide substitutions in 100 nt.

in the molar ratio 1:0.9:2:2, which brings the position of the second molecule of glutamic acid into question (one molecule is known to be at position 2 of the peptide subunit). When analysed in our laboratory, the hydrolysate (120 °C, 6 M HCl, 16 h) of the purified murein revealed that the ratio of L-lysine:aspartic acid:glutamic acid:alanine was 1:0.9:1:2 (Groth *et al.*, 1999). Analysis of partial hydrolysis fragments (Schleifer & Kandler, 1972) indicated the presence of the peptide L-Lys-Asp. Thus, the A4 α type is of the variation A11.31 (DSMZ, 2001).

The topologies of 16S rDNA dendrograms recovered by different phylogenetic methods suggest that *C. variabile* is not an authentic member of the genus *Cellulosimicrobium*. However, as already discussed in the paper that excluded *C. cellulans* from the genus *Cellulomonas* (Schumann *et al.*, 2001), branching points should not be used as the sole criterion for reclassification purposes, inasmuch as the positions of *C. cellulans* and *C. variabile* are not supported by bootstrap values of high significance (Fig. 1). The rationale for the reclassification of *C. variabile* as the nucleus of a novel genus adjacent to the genera *Xylanimonas* and *Promicromonospora* is also based on the presence of the peptidoglycan type L-Lys-Asp, which is found in *Xylanimonas cellulosilytica*, but not in strains of *C. cellulans* (L-Lys-Ser-Asp, type A4 α , variation A11.36) or members of the genus *Promicromonospora* (L-Lys-Ala, type A3 α , variation A11.4; see footnote to Table 1). Also, although

C. variabile and *C. cellulans* both possess galactose, rhamnose and glucose in their whole-cell hydrolysates, *C. cellulans* also possesses fucose and mannose. Another distinguishing feature is the composition of predominant fatty acid methyl esters: *C. variabile* contains C_{14:0} and ai-C_{17:0}, which are absent in *C. cellulans*. Polar lipids, determined according to the method of Minnikin *et al.* (1979) and Collins & Jones (1980), are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and two unknown phospholipids. This composition differs only quantitatively from that found in *C. cellulans*. However, differences in fatty acid and polar lipid compositions and whole-cell sugars should not be overvalued and these properties are mainly used for the differentiation of species, not of genera.

The nearest neighbours of *C. variabile* as determined to date are *Xylanimonas cellulosilytica* and *Xylanibacterium ulmi*, cellulase- and xylan-hydrolysing organisms from *Ulmus nigra*, and *P. pachnodae* (Cazemier *et al.*, 2003), a (hemi)-cellulolytic and xylanolytic bacterium that was isolated from the hindgut of larvae of the herbivorous beetle *Pachnoda marginata*. Chemotaxonomic data and thorough phenotypic analysis of the latter prokaryotic organism are lacking. Based on the evidence indicated in Table 1, we propose to reclassify *C. variabile* as the type species of a novel genus, for which the name *Isoptericola variabilis* Bakalidou *et al.* 2002 gen. nov., comb. nov. is proposed. The name has been chosen to depict that *C. variabile* was isolated from the hindgut of the Australian termite *Mastotermes darwiniensis* (Froggatt).

Description of *Isoptericola* gen. nov.

Isoptericola (I.sop.te.ri'co.la. N.L. n. *Isoptera* order of termites; L. masc. suffix *-cola* inhabitant; N.L. masc. n. *Isoptericola* inhabitant of termites).

The following description is based on the description of the species *Cellulosimicrobium variabile* DSM 10177^T (Bakalidou *et al.*, 2002) and our own data (peptidoglycan).

Cells are rod-shaped, V-shaped or coccoid and non-motile. Primary mycelium is formed. Gram-positive. Murein contains the amino acids lysine, aspartic acid, glutamic acid and alanine in a molar ratio of 1:0.9:1:2 and belongs to the peptidoglycan type A4 α , variation A11.31. N-Glycolylmuramic acid, mycolic acids and hydroxy fatty acids are absent. Whole-cell sugars are galactose, rhamnose and glucose (ratio 4:2:1). Main menaquinone is MK9(H₄). Major fatty acids are ai-C_{15:0} (53.6%), i-C_{15:0} (17%), C_{16:0} (7.2%), i-C_{16:0} (6.0%), ai-C_{17:0} (7.0%) and C_{14:0} (6.6%). i-C_{14:0} (1.5%), i-C_{17:0} (0.6%) and C_{15:0} (0.5%) occur in smaller amounts. Phospholipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and two unknown phospholipids. Cellulolytic and xylanolytic; facultatively anaerobic; acid is produced from some carbohydrates. DNA G+C content is 70–72 mol% (nuclease P1 digest, HPLC). Phylogenetically related to

Table 1. Chemotaxonomic characteristics that differentiate strains of the genera *Cellulosimicrobium*, *Xylanimonas* and *Promicromonospora*

Taxa: 1, *C. variabile* DSM 10177^T [data from Bakalidou *et al.* (2002)]; 2, *C. cellulans* DSM 43879^T [data from Stackebrandt & Keddie (1986) and Schumann *et al.* (2001)]; 3, *Xylanimonas cellulosilytica* XIL07^T [data from Rivas *et al.* (2003)]; 4, *P. pachnodae* DSM 12657^T; 5, authentic *Promicromonospora* species [data from Busse *et al.* (2003)]. +, Positive; w, weak; –, negative; DPG, diphosphatidylglycerol; GL, unknown glycolipid; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipid; ND, not determined.

Property	1	2	3	4	5
Shape	Short rods, V-shaped or coccoid	Short rods	Coccoid	Irregularly shaped, single or in pairs	Short rods, V- or Y-shaped or coccoid
Mycelium	Primary mycelium	Primary mycelium	–	–	Mycelium
Aerial hyphae	–	–	–	–	+
Fermentation	+	+	w	+	Rare
Peptidoglycan composition	L-Lys–D–Asp	L-Lys–D–Ser–D–Asp	L-Lys–D–Asp	L-Lys–L–Ser–D–Glu	Lys–Ala–Glu*
Cell-wall sugars	Rha, Gal, Glu	Rha, Fuc, Gal	Gal, Rha	Rha, Gla, Glc	Glc, Gal, Xyl or Glc, Gal, Rha
Phospholipid composition	PG, DPG, PI, PL	PG, DPG, PI, PL	PG, DPG, PI, PIM	PG, DPG, PI, PIM, PL	PG, DPG, PGL, PL, GL
Major menaquinones	MK9(H ₄)	MK9(H ₄)	MK9(H ₄), MK8(H ₄)	ND	MK9(H ₄)
Predominant fatty acids	ai-C _{15:0} , i-C _{15:0} , C _{14:0} , C _{16:0} , i-C _{16:0} , ai-C _{17:0}	ai-C _{15:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{15:0} , i-C _{16:0} , C _{16:0}	ai-C _{15:0} , i-C _{15:0}

*Conflicting reports on the peptidoglycan type are given in the literature: L-Lys–Ala (Kalakoutskii *et al.*, 1989) and Lys–Ala–Glu (Evtushenko *et al.*, 1984; P. Schumann, unpublished data).

Xylanimonas cellulosilytica, *Xylanibacterium ulmi* and *Promicromonospora pachnodae*. The type species is *Isoptericola variabilis* Bakalidou *et al.* 2002.

Description of *Isoptericola variabilis* Bakalidou *et al.* 2002 corrig., comb. nov.

Isoptericola variabilis (va.ri.a'bi.lis. L. masc. adj. *variabilis* variable, as cells can be rods or cocci).

The description is the same as that given by Bakalidou *et al.* (2002) with the modification of the amino acid composition of the murein, as given in the genus description.

The type strain is MX5^T (=DSM 10177^T=ATCC BAA-303^T).

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