

Cellulosimicrobium variabile* sp. nov., a cellulolytic bacterium from the hindgut of the termite *Mastotermes darwiniensis

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A novel cellulolytic and xylanolytic bacterium, strain MX5^T, was isolated from the hindgut contents of the Australian termite *Mastotermes darwiniensis* (Froggatt). The isolate was a facultative anaerobe and had a Gram-positive cell-wall profile. The rod-shaped bacterium formed irregular coryneform and coccoid cells during growth. Phylogenetic analysis of the 16S rDNA provided evidence that the organism was closely related to the as-yet undescribed cellulolytic strain SR272 and the non-validly described species '*Cellulomonas pachnodae*' as well as *Promicromonospora citrea* and *Promicromonospora sukumoe*. Strain MX5^T was assigned to the genus *Cellulosimicrobium* on the basis of phylogenetic and chemotaxonomic criteria. The murein of strain MX5^T contained the diamino acid lysine. *N*-Glycolylmuramic acid, mycolic acids and hydroxy fatty acids were absent. The major neutral sugar in the cell wall was galactose and the major quinone was menaquinone MK-9(H₄). The predominant fatty acids were ai-C15:0, i-C15:0, i-C16:0 and C16:0. The G+C content of the DNA was in a range 70–72 mol%. On the basis of 16S rDNA sequence similarities and chemotaxonomic features, MX5^T was clearly different from *Cellulosimicrobium cellulans* and other validly described species within this phylogenetic group. For this reason, a novel species is described, for which the name *Cellulosimicrobium variabile* sp. nov. is proposed.

Keywords: *Cellulosimicrobium*, *Cellulomonas*, termites, intestinal flora, cellulose degradation

INTRODUCTION

Termites play an important role in the turnover of lignocellulolytic material. Although endogenous cellulases are active (Veivers *et al.*, 1982; Tokuda *et al.*, 1997; Watanabe *et al.*, 1998), the microflora also has a significant impact on cellulose degradation (Breznak, 1982; Breznak & Brune, 1994; Varma *et al.*, 1994). Symbiotic flagellates are major contributors to cellulose decomposition in the guts of lower termites (Cleveland, 1924; Gutierrez, 1956; Hogan *et al.*, 1988;

Hungate, 1943; Odelson & Breznak, 1985; Trager, 1934), whereas fungus-growing termites can also consume the fungal cellulases together with fungus nodules (Rouland *et al.*, 1988).

Because many attempts to isolate cellulose degraders were unsuccessful (Cleveland, 1924; Dickman, 1931; Hungate, 1936; Schultz & Breznak, 1978), it has been concluded that bacteria are not important contributors to cellulose digestion in termites (Slaytor, 1992), and only a few cellulose-degrading bacteria have been isolated, from a few termite species (Hethener *et al.*, 1992; Hungate, 1946; Mannesmann, 1972; Pasti & Belli, 1985; Paul *et al.*, 1986; Saxena *et al.*, 1993; Sebald & Prévot, 1962; Thayer, 1976).

In this study, we have identified and characterized a novel cellulolytic and xylanolytic bacterium, strain MX5^T, from the Australian termite *Mastotermes*

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Abbreviations: pNA, *para*-nitroanilide; pNP, *para*-nitrophenyl.

The EMBL accession numbers for the 16S rDNA sequences of strains MX5^T, KHC4, KMaC5 and KNeC1 are respectively AJ298873, AJ315029, AJ296274 and AJ313329.

darwiniensis. This organism was characterized by a polyphasic approach. On the basis of a phylogenetic analysis of the 16S rDNA, the isolate is related to a clade containing *Cellulomonas*, *Cellulosimicrobium* and *Promicromonospora* species (Schumann *et al.*, 2001). On the basis of these and chemotaxonomic data, a novel species is proposed, *Cellulosimicrobium variabile* sp. nov.

METHODS

Termites. The termites *Heterotermes indicola* (Wasman), *Mastotermes darwiniensis* (Froggatt), *Nasutitermes nigriceps* (Haldeman), *Neotermes castaneus* (Burmeister), *Schedorhinotermes intermedius* (Isolieser) and *Zootermopsis angusticollis* (Hagen) were obtained from the Bundesanstalt für Materialforschung und Materialprüfung (BAM, Berlin, Germany).

Micro-organisms and culture conditions. The isolation of the novel strain MX5^T from gut contents of *Mastotermes darwiniensis* (Froggatt) on xylan-containing agar plates was described by Schäfer *et al.* (1996). *Rhodococcus rhodochrous* DSM 43241^T and *Cellulosimicrobium (Cellulomonas) cellulans* DSM 43879^T were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). *Pseudomonas aeruginosa* strain KNB5 was isolated from the gut contents of *Nasutitermes nigriceps* (Haldeman). *Bacillus cereus* strain KD12 (Kuhnigk & König, 1997) was obtained from the gut contents of *Reticulitermes santonensis* (Feytand). Bacteria were grown in tryptic soy broth (TSB; Difco) or on tryptic soy agar (TSA; Difco) at 28 °C. Cellulose degradation was tested on agar plates at 28 °C using the medium described by Yamin (1978) containing, in addition, 15 g agar l⁻¹, 10 g carboxymethylcellulose l⁻¹ (instead of glucose) and 0.5 g yeast extract l⁻¹ and supplemented with vitamins and trace minerals (Balch *et al.*, 1979). Anaerobic growth was tested in the latter medium without agar containing glucose (10 g l⁻¹) instead of carboxymethylcellulose and supplemented with a resazurin solution (1 g l⁻¹; 1 ml stock solution l⁻¹) as redox indicator and Na₂S (0.1 g l⁻¹). The gas phase was N₂/CO₂ (80:20; v/v).

Microscopy. Cells were observed and photographed with a phase-contrast microscope (Axioskop; Zeiss) equipped with a Plan-Neofluar objective (100/1.3, oil) and a camera (MC 63). Transmission electron microscopy was performed as described previously (Breunig *et al.*, 1993).

Physiological tests. Physiological tests were performed as described elsewhere (Kämpfer *et al.*, 1991). In addition, BioLog GN MicroPlates and the MicroLog computer software (BioLog identification system; Biolog Inc.) were used.

Cell wall components. After two cycles of washing (9 g NaCl l⁻¹; cells collected at 4000 g, 10 min at 4 °C in an Eppendorf centrifuge ZK 401 BHG), the cells from a 500 ml culture were disrupted by two cycles through a French press (137 MPa; American Instrument Company). Residual intact cells were separated by centrifugation (4000 g; 10 min; 4 °C). Crude cell walls were harvested from the supernatant by centrifugation (45 000 g; 25 min, 4 °C, Beckman L8-60 M). The crude cell walls were suspended in 40 g SDS l⁻¹ and heated at 100 °C for 20 min. The cell walls were pelleted by centrifugation (45 000 g; 25 min, 20 °C) and washed three times with water before a final centrifugation step (45 000 g; 25 min, 20 °C) to give the cell wall preparation.

For murein analysis, the cell walls were hydrolysed with 6 M HCl at 100 or 120 °C for 18 h. The murein constituents were determined with an amino acid analyser (Biotronic LC 5001) programmed for murein analysis (Biotronic). For analysis of neutral sugars, the cell walls were hydrolysed with 0.5 M H₂SO₄ at 100 °C for 2 h. The neutral sugars were separated on a Carbowax PA1 column (Dionex) at 20 °C. NaOH (12 mM) was used as eluant at a rate of 1 ml min⁻¹. The elution profile was recorded amperometrically (PAD; Dionex).

In some bacteria, glycolylmuramic acid can replace acylmuramic acid. For the detection of glycolic acid, 20 mg freeze-dried cells of isolate MX5^T and *Rhodococcus rhodochrous* DSM 43241^T (standard) were hydrolysed (4 M HCl, 20 h, 100 °C) and further treated as described by Kutzner *et al.* (1986). The development of a purple colour after addition of a 2,7-naphthoindole solution was taken as indicative of the presence of glycolic acid.

Mycolic acids. For the detection of mycolic acids and hydroxy fatty acids, freeze-dried cells of isolate MX5^T and standards *Rhodococcus rhodochrous* DSM 43241^T (mycolic acids C₃₅-C₅₅; R_f = 0.36), *Pseudomonas aeruginosa* (2-hydroxy fatty acid methyl esters, R_f = 0.16; 3-hydroxy fatty acid methyl esters, R_f = 0.22) and *Bacillus cereus* (fatty acid methyl esters, R_f = 0.84) (Kutzner *et al.*, 1986) were used. After transesterification of the fatty acids, the corresponding methyl ester derivatives were extracted with *n*-hexane. The fatty acid methyl esters were separated on activated (20 min, 120 °C) silica G60 plates. A mixture of petrolether and diethylether (85:15, v/v) was used as eluant. Compounds were detected with molybdate reagent (Merck).

Quinones. Menaquinones were extracted from 100 mg freeze-dried cells of isolate MX5^T or *Cellulosimicrobium cellulans* DSM 43879^T (standard) as described by Kutzner *et al.* (1986). Menaquinones were separated on silica GF 60 plates using a mixture of petrolether and diethylether (85:15, v/v) as eluant. The UV-positive bands were eluted with diethylether. The eluted menaquinones were further separated on a Nucleosil 100-5 C18 column (Macherey-Nagel) at 40 °C. The eluant was a mixture of acetonitrile and 2-propanol (60:35, v/v). The elution rate was 1 ml min⁻¹. An additional separation was performed on a Nucleosil 100-10 SA column (Macherey-Nagel) at 50 °C with methanol at a flow rate of 1 ml min⁻¹. With the latter column, menaquinones can be separated according to the position of the double bond. The elution profiles were recorded at 254 nm.

Phospholipids. Phospholipids were extracted from freeze-dried cells as described by Kutzner *et al.* (1986) and separated on silica G60 plates. Chloroform/methanol/water (65:25:4, by vol.) and chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) were used as eluants. Detection of glycolipids was carried out with anisaldehyde, ninhydrin, α -naphthol and molybdate reagents (Kutzner *et al.*, 1986).

Fatty acids. Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID, Inc.). Extracts were analysed by using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described previously (Kämpfer & Kroppenstedt, 1996).

Determination of the G + C content of the DNA. Genomic DNA was isolated as described by Johnson (1994). The DNA was digested with P1 nuclease and the nucleotides were separated on a Nucleosil NH₂ column (300 × 4 mm; Macherey-Nagel). Potassium phosphate buffer (25 mM),

pH 3-4, was used at an elution rate of 1.5 ml min⁻¹. DNA from calf thymus (G + C = 42 mol%) and from *Escherichia coli* (G + C = 50 mol%) served as standards.

Amplification, cloning and sequencing of 16S rDNA. Genomic DNA was isolated with InstaGene DNA purification matrix (Bio-Rad). The primers Eubak5 and Eubak3 (Berchtold *et al.*, 1994) were used for 16S rRNA gene amplification (30 cycles: 94 °C, 1 min; 55 °C, 1 min; 72 °C, 2 min) resulting in nearly full-length 16S rDNA amplification products. The amplified DNA was checked by agarose gel electrophoresis and purified with the QIAquick PCR purification kit (Qiagen). The purified PCR products were ligated into pBluescript SK(+) (Stratagene) and cloned in *E. coli* K-12 DH5 α . The plasmid was isolated with the QIAquick plasmid purification kit (Qiagen). Sequencing of the 16S rRNA gene was carried out as described by Berchtold & König (1996).

Phylogenetic analysis. The resulting 16S rDNA sequence was compared to sequences from the RDP (Maidak *et al.*, 2000) and EMBL (Baker *et al.*, 2000) databases to find the most closely related species. CLUSTAL W (Thompson *et al.*, 1994) was used to align the MX5^T sequence in the prokaryotic 16S rRNA alignment from the RDP (Maidak *et al.*, 2000) followed by manual checking of the resulting alignment. A dataset of 26 sequences with 1458 nucleotide positions that could be aligned unambiguously was used for the construction of phylogenetic trees by distance matrix (Fitch & Margoliash, 1967; Saitou & Nei, 1987), parsimony and maximum-likelihood methods (Felsenstein, 1981) with DNADIST, NEIGHBOR, FITCH, DNAPARS and DNAML from the PHYLIP package (Felsenstein, 1993). Tree topologies were evaluated with bootstrap analyses (Felsenstein, 1985) using SEQBOOT from the PHYLIP package.

RESULTS AND DISCUSSION

Morphology

Isolate MX5^T stained Gram-positive and possessed a typical Gram-positive wall structure with a thickness of about 20 nm (Fig. 1). Cultures were composed of

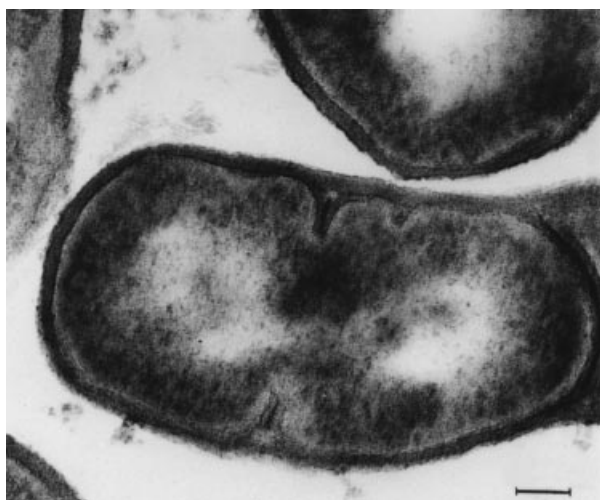


Fig. 1. Electron micrograph of thin sections of strain MX5^T. Ultrathin sections were stained with Reynold's lead citrate solution. Bar, 0.06 μ m.

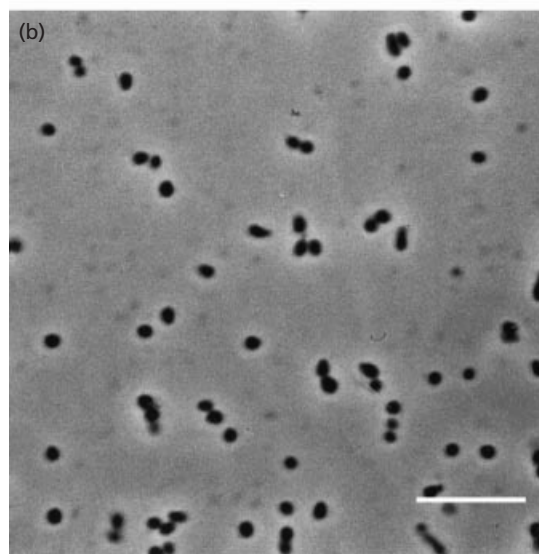
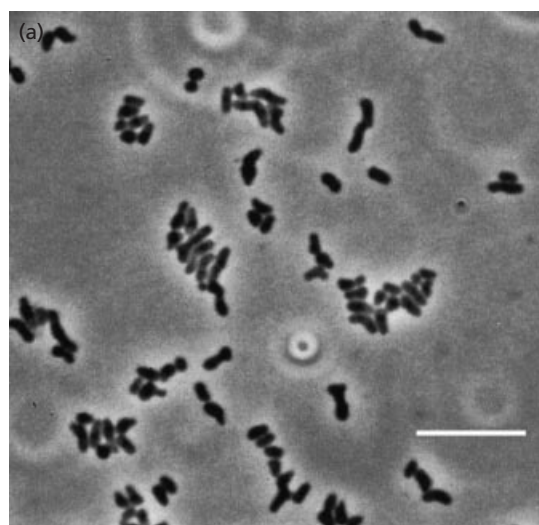


Fig. 2. Phase-contrast micrographs of strain MX5^T showing the formation of irregular coryneform rod-shaped cells (culture time 48 h) (a) and coccoid cells (culture time 72 h) (b). Bars, 2 μ m.

rod-shaped cells (about 0.3 \times 0.7 μ m; Fig. 1) or V-shaped cells and formed cocci (about 0.25 μ m in diameter) after 3 days (Fig. 2). Spores and motility were not observed. Cells grew as smooth, yellow colonies on TSA with a diameter of about 2 mm after 48 h at 28 °C.

Physiological characteristics

The isolation and xylanase production of isolate MX5^T were described by Schäfer *et al.* (1996). We found that strain MX5^T also formed clear zones on carboxymethylcellulose-containing agar plates, indicating the production of cellulolytic activities. Strain MX5^T also grew anaerobically. The isolate showed acid production from various sugars and related com-

pounds and several sugars were also utilized as sole sources of carbon (see below). Only a few organic acids, including amino acids, were utilized (for details, see species description below). Most of the tested *p*-nitrophenyl (pNP) derivatives were hydrolysed (see below).

G + C content of the DNA

The G + C content of the DNA of strain MX5^T was within the range 70–72 mol%.

Cell wall composition

The peptidoglycan of isolate MX5^T was composed of the amino acids lysine, aspartic acid, glutamic acid and alanine in a molar ratio of 1.0:0.9:2.0:1.8. When the cell walls were hydrolysed at 100 °C, partial hydrolysis products contained ϵ -(aminosuccinyl)-lysine (Schleifer & Kandler, 1972), indicating the presence of peptidoglycan type A4 α . For complete hydrolysis, 120 °C was required. Glycolic acid and mycolic acids were not detected. The main neutral sugar of the cell walls was galactose. The neutral sugars galactose, rhamnose and glucose were found in a molar ratio of 4:2:1.

Components of the cytoplasmic membrane

Hydroxy fatty acids were detected in *Pseudomonas aeruginosa* (used as a reference standard), but were not found in isolate MX5^T. The major lipids detected were phosphatidylinositol mannoside, phosphatidylinositol and phosphatidylethanolamine. The major menaquinone of isolate MX5^T co-migrated under all the chromatography systems tested with the major menaquinone of *Cellulosimicrobium cellulans* DSM 43879^T (used for comparison), which was menaquinone MK-9(H₄) (Stackebrandt & Keddie, 1986).

Strain MX5^T contained the fatty acids ai15:0 (53.6 mol%), i15:0 (17.0 mol%), 16:0 (7.2 mol%), i16:0 (6.0 mol%), ai17:0 (7.0 mol%), 14:0 (6.6 mol%), i14:0 (1.5 mol%), i17:0 (0.6 mol%) and 15:0 (0.5 mol%). A similar fatty acid profile has been described for *Cellulosimicrobium cellulans* DSM 43879^T (Funke *et al.*, 1995).

Assignment of isolate MX5^T to *Cellulosimicrobium*

The partial 16S rDNA sequence (1472 bp) of strain MX5^T was aligned with the sequences of 25 other related strains obtained from the RDP (Maidak *et al.*, 2000). The alignment was used for phylogenetic analysis with distance-matrix, parsimony and maximum-likelihood methods. All trees obtained were nearly identical to the maximum-likelihood tree depicted in Fig. 3. The tree shows that strain MX5^T clustered together with two strains from the EMBL nucleotide sequence database (strain SR272, 99% sequence similarity; '*Cellulomonas pachnodae*' strain VPCX2, 97% sequence similarity). In addition, the rRNA of both *Promicromonospora citrea* DSM 43110^T

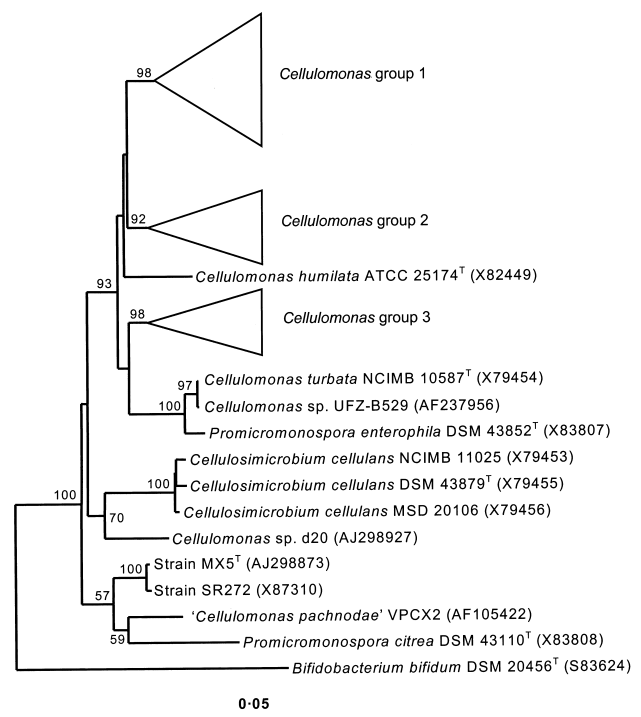


Fig. 3. Phylogenetic tree reflecting the relationship of strain MX5^T and other cellulomonads. The tree is based on the results of a maximum-likelihood analysis of 16S rDNA sequences (1458 alignment positions). Bootstrap values above 50% are indicated at branch-points. For tree construction, the 16S rDNA sequences of additional cellulomonads were included: group 1, *Cellulomonas uda* DSM 20107^T (X79457), *Cellulomonas gelida* DSM 20111^T (X79461), *Cellulomonas iranensis* ATCC 700643^T (AF064702), *Cellulomonas persica* ATCC 700642^T (AF064701), *Cellulomonas sp.* 794 (Y09656), *Cellulomonas flavigena* DSM 20109^T (X79463); group 2, *Cellulomonas fimi* DSM 20113^T (X79460), *Cellulomonas biazotea* DSM 20112^T (X79462), *Cellulomonas cellasea* DSM 20118^T (X79459), *Cellulomonas fermentans* DSM 3133^T (X79458); group 3, *Cellulomonas sp.* 1781 (Y09659), *Cellulomonas sp.* CE40 (X82598), *Cellulomonas hominis* DSM 9581^T (X82598). The position of *Promicromonospora sukumoe* (AJ272024) in the phylogenetic tree is close to *Promicromonospora citrea* (bootstrap value, 100%) (Schumann *et al.*, 2001). Bar, 5% estimated sequence divergence.

and *Cellulosimicrobium cellulans* DSM 43879^T showed 96% similarity to MX5^T.

On the basis of the Gram-staining characteristics, cell-wall composition and G + C content, the facultative anaerobic isolate MX5^T was assigned to the actinomycete branch of the Gram-positive bacteria.

N-Glycolylmuramic acid is found in coryneforms with murein type B (*Aureobacterium*, *Microbacterium*), in actinomycetes possessing mycolic acids (*Mycobacterium*, *Nocardia*, *Rhodococcus*) and among the *Actinoplanaceae* and *Micromonosporaceae*. The absence of both glycolic acid and mycolic acids indicated that isolate MX5^T did not belong to these genera.

The presence of cellulolytic activities and cell walls composed of the sugars galactose, rhamnose and

glucose indicated that the organism belonged to one of the families *Cellulomonadaceae* and *Promicromonosporaceae*.

Strain MX5^T, together with the as-yet undescribed strain SR272, formed a separate subgroup, clustering close to the *Cellulosimicrobium* branch of the family *Cellulomonadaceae* (Rainey *et al.*, 1995; Schumann *et al.*, 2001). Therefore, we propose to assign isolate MX5^T to a novel species of the genus *Cellulosimicrobium*, *Cellulosimicrobium variabile* sp. nov. The rationale for the assignment of the novel isolate MX5^T to the genus *Cellulosimicrobium* rather than *Cellulomonas* or *Promicromonospora* was based on distinguishing phenotypic features in addition to 16S rRNA sequence data. Like *Cellulosimicrobium* strains (Stackebrandt *et al.*, 1978), strain MX5^T possesses the peptidoglycan type A4 α (with L-lysine as diamino acid and aspartate in the interpeptide bridge). This feature is not found in strains of authentic *Cellulomonas* species, which possess ornithine as diamino acid, with the exception of *Cellulomonas humilata* ATCC 25174^T (Collins & Pascual, 2000), which contains both ornithine and lysine. Both *Cellulosimicrobium cellulans* DSM 43879^T and strain MX5^T contain galactose in their cell walls. In contrast, most authentic *Cellulomonas* species, with the exception of *Cellulomonas biazotea*, do not contain galactose.

Isolate MX5^T possesses 16S rDNA sequence similarity of 95–96% to species of the related genus *Promicromonospora*, but can be distinguished from this genus by phenotypic features. The species of the genus *Promicromonospora* form persistent branching substrate and aerial mycelia which can undergo fragmentation. *Promicromonospora citrea* and *Promicromonospora sukumoe* are strict aerobes, while *Promicromonospora enterophila* is a facultative anaerobe. None of the three *Promicromonospora* species possesses an A4 α -type peptidoglycan with aspartic acid in the interpeptide bridge (Kalakoutskii *et al.*, 1989; Takakashi *et al.*, 1987; Schumann *et al.*, 2001).

Interestingly, the closely related non-validly described species '*Cellulomonas pachnodae*' strain VPCX2 was isolated from the gut of the rose-chaffer *Pachnoda marginata*, which, like termites, feeds on cellulose-rich plant material (Cazemier *et al.*, 1999). Strain VPCX2 also possesses cellulolytic activity. Whether this indicates that this phylogenetic group is specially adapted to the gut systems of plant-feeding insects remains to be examined.

In conclusion, according to the available 16S rDNA sequence data, MX5^T does not possess a sequence similarity of more than 96% to any validly described species within the radiation of the *Cellulosimicrobium* and *Cellulomonas* lineages. Organisms with less than 97% 16S rDNA sequence similarity possess a DNA–DNA reassociation of less than 60% (Stackebrandt & Goebel, 1994; Fröhlich *et al.*, 1999). In addition, there are several phenotypic features that allow the clear differentiation of MX5^T from *Cellulosimicrobium*

cellulans (Table 1). On the basis of these results, a novel species is described, for which the name *Cellulosimicrobium variabile* sp. nov. is proposed.

Distribution of cellulomonads in the termite gut

Strains related to *Cellulomonas cartae* (reclassified as *Cellulosimicrobium cellulans*; Schumann *et al.*, 2001) (100% 16S rDNA sequence similarity; 400–450 nucleotides) were isolated from *Mastotermes darwiniensis* (strain KMaC5), *Neotermes castaneus* (strain KNeC1) and *Heterotermes indicola* (strain KHC4). Other strains showing the same 16S rDNA restriction fragment length pattern with restriction enzymes *Bsu*RI and *Hpa*II (*Bsu*RI: 290–220–150; *Hpa*II: 600–180) as the above-mentioned *Cellulosimicrobium cellulans*-related strains were obtained from *Mastotermes darwiniensis* (strain X6), *Schedorhinotermes intermedius* (strains KScC2, KScC6), *Nasutitermes nigriceps* (strain NA2) and *Zootermopsis angusticollis* (strain ZC1). Strain KScC3 from *Schedorhinotermes intermedius* had the same 16S rDNA restriction fragment length pattern (*Bsu*RI: 310–240–150; *Hpa*II: 480–200) as *Cellulosimicrobium variabile* strain MX5^T. This indicates that *Cellulosimicrobium*-related strains are widespread in termites.

Description of *Cellulosimicrobium variabile* sp. nov.

Cellulosimicrobium variabile (va.ri.a'bi.le. L. neut. adj. *variabile* variable, because the cells form rods or cocci).

Cells are rod-shaped (0.3 × 0.7 μ m), V-shaped or coccoid (diameter 0.25 μ m) and non-motile. Cells stain Gram-positive. Yellow, smooth colonies on TSA (Difco) about 2 mm in diameter after 48 h at 28 °C. Facultatively anaerobic. Cellulolytic and xylanolytic activities are produced. The murein contains the amino acids lysine, aspartic acid, glutamic acid and alanine in a molar ratio of 1.0:0.9:2.0:1.8 and belongs to the peptidoglycan type A4 α . *N*-Glycolylmuramic acid, mycolic acids and hydroxy fatty acids are absent. The neutral sugars galactose, rhamnose and glucose are found in a molar ratio of 4:2:1. The main quinone is menaquinone MK-9(H₄). Major fatty acids are ai15:0 (53.6 mol%), i15:0 (17.0 mol%), 16:0 (7.2 mol%), i16:0 (6.0 mol%), ai17:0 (7.0 mol%), 14:0 (6.6 mol%), i14:0 (1.5 mol%), i17:0 (0.6 mol%) and 15:0 (0.5 mol%). Acid is produced from D-glucose, L-arabinose, raffinose, L-rhamnose, D-xylose, D-cellobiose, D-melibiose, D-arabitol and D-mannose. No acid production is observed from sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, maltose, methyl β -D-glucoside or erythritol. The following substrates are used as sole sources of carbon: *N*-acetyl D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-maltose, D-mannose, D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-trehalose, maltitol, mannitol, sorbitol, acetate, fumarate (weak), DL-hydroxybutyrate, pyruvate, L-histidine and L-proline. *N*-Acetyl D-

Table 1. Phenotypic characteristics that differentiate strains of the genera *Cellulosimicrobium* and *Cellulomonas*

Strains: 1, MX5^T; 2, *Cellulosimicrobium cellulans* DSM 43879^T; 3, *Cellulomonas fermentans* DSM 3133^T; 4, *Cellulomonas humilata* ATCC 25174^T; 5, *Cellulomonas hominis* DSM 9581^T; 6, *Cellulomonas persica* ATCC 700642^T; 7, *Cellulomonas iranensis* ATCC 700643^T; 8, *Cellulomonas biazotea* DSM 20112^T; 9, *Cellulomonas cellasea* DSM 20118^T; 10, *Cellulomonas flavigena* DSM 20109^T; 11, *Cellulomonas fimi* DSM 20113^T; 12, *Cellulomonas gelida* DSM 20111^T; 13, *Cellulomonas uda* DSM 20107^T. Phenotypic characteristics of described species are from Elberson *et al.* (2000), Funke *et al.* (1995), Kämpfer *et al.* (1993), Schaal (1986) and Stackebrandt & Keddie (1986). Abbreviations: ND, not determined; +, positive; ++, strongly positive; -, negative; Lys, L-lysine; Orn, L-ornithine; Y, yellow; Y-W, yellow-white; W, white.

Characteristic	<i>Cellulosimicrobium</i>				<i>Cellulomonas</i>								
	1	2	3	4	5	6	7	8	9	10	11	12	13
Cell wall diamino acid	Lys	Lys	Orn	Lys/Orn	Orn	Orn	Orn	Orn	Orn	Orn	Orn	Orn	Orn
Cell wall composition:													
Galactose	++	+	-	-	ND	-	-	+	-	-	-	+	-
Glucose	+	-	++	+	ND	+	+	-	-	+	+/-	+	+
Glucosamine	-	-	-	-	ND	++	++	++	-	++	++	++	+
Mannose	+	-	-	-	ND	+	+	-	+	+	-	-	-
Rhamnose	+	++	+	++	ND	++	++	++	++	-	++	-	-
Fucose	-	+	-	+	-	-	-	-	-	-	+	+	-
Fermentation of:													
Glucose	+	+	+	+	+	ND	ND	+	+	+	+	+	+
Maltose	-	+	+	+	+	ND	ND	+	+	+	+	+	+
Sucrose	-	+	+	+	+	ND	ND	+	+	+	+	+	+
Mannitol	-	-	+	+	-	ND	ND	-	+	-	-	-	-
Xylose	+	+	+	+	+	ND	ND	+	+	+	+	+	+
Dextrin	ND	+	-	+	+	+	+	-	-	+	+	+	+
Methyl β -xyloside	-	+	-	ND	+	ND	ND	-	-	-	+	-	-
Rhamnose	+	-	-	+	+	ND	ND	+	-	-	+	-	-
Methyl α -mannoside	ND	-	-	ND	+	ND	ND	-	-	-	-	-	-
Growth substrates:													
Acetate	+	+	ND	-	ND	+	+	+	+	+	-	+	+
Dextrin	ND	+	ND	ND	ND	+	+	-	-	+	+	+	+
Gluconate	+	+	ND	+	ND	-	-	-	-	+	-	-	-
Lactate	+	+	ND	-	ND	-	-	+	+	-	+	-	-
Raffinose	ND	-	ND	ND	ND	-	-	+	-	-	-	-	-
Mannose	+	+	ND	ND	ND	+	+	+	+	+	+	+	+
D-Ribose	+	+	ND	ND	ND	-	-	-	-	+	-	-	-
Motility	-	ND	-	-	+	+	+	+	-	-	+	+	-
Colony colour	Y-W	Y-W	W	W	Y-W	Y-W	Y-W	Y-W	Y	Y	Y-W	Y-W	W

galactosamine, adonitol, inositol, putrescine, propionate, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, glutarate, itaconate, L-malate, mesaconate, suberate, L-alanine, β -alanine, L-leucine, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate are not utilized. Hydrolysis of the following chromogenic substrates is positive: aesculin, pNP β -D-galactopyranoside, pNP α -D-glucopyranoside, pNP β -D-glucopyranoside, pNP β -xylopyranoside, bis-pNP phosphate, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxy-5'-pNP phosphate, L-alanine *para*-nitroanilide (pNA) and L-proline-pNA. pNP β -D-glucuronide is not hydrolysed. The G + C content of the DNA is 70–72 mol %. The 16S rDNA sequence of isolate MX5^T (accession number AJ298873) has high similarity to those of two as-yet undescribed

isolates, strain SR 272 (99%; accession number X87317) and strain VPCX2 (*Cellulomonas pachnodae*; host, larvae of the rose-chaffer *Pachmoda marginata*) (97%; accession number AF105422) from the EMBL nucleotide sequence database. Strains can be distinguished from *Cellulosimicrobium cellulans* and from *Cellulomonas* species by the phenotypic features compiled in Table 1.

Habitat: hindgut contents of the Australian termite *Mastotermes darwiniensis* (Froggatt). The type strain is MX5^T (= DSM 10177^T = ATCC BAA-303^T).

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