**Clostridium termitidis** sp. nov., a Cellulolytic Bacterium from the Gut of the Wood-feeding Termite, *Nasutitermes lujiae*

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**Summary**

An anaerobic, mesophilic, spore-forming, cellulolytic bacterium was isolated from the gut of a wood-feeding termite, *Nasutitermes lujiae*. The cells were Gram-positive rods, motile by peritrichous flagella, and formed oval terminal spores which swelled the cells. The deep colonies were circular, punctiform and slightly yellow; the surface colonies spread rapidly and were diffuse. Cellulose, cellobiose, glucose, fructose, galactose, lactose, mannose, ribose, sorbose, xylose, maltose, melibiose, mannitol and sorbitol served as carbon source; glycerol was poorly used. The fermentation products were acetate, ethanol, H₂, CO₂. Optimal growth occurred at 37°C and at pH 7.5. The deoxyribonucleic acid composition was 39.2 mol% guanine plus cytosine. The name *Clostridium termitidis* sp. nov. is proposed. The type strain is strain CT1112 (= DSM 5398).

**Key words:** *Clostridium termitidis* sp. nov. – Cellulose – Mesophilic – Wood-feeding termites – *Nasutitermes lujiae* – Intestinal tract

**Introduction**

During the last decade, the potential for converting cellulolic wastes into industrial substrates has stimulated current interest in cellulose fermentation. Many cellulolytic microorganisms have been isolated from various biotopes, particularly those belonging to the genus *Clostridium* from the rumen (Hungate, 1944; Van Gylswuy et al., 1980; Kelly et al., 1987; Varel, 1989), anaerobic digestors (Ng et al., 1977; Sleat et al., 1984; Sleat and Mah, 1985; Palop et al., 1989; Yang et al., 1990), compost (Madden, 1983; Petitdemange et al., 1984; Sukhnumavasi et al., 1988; Yanling et al., 1991) estuarine sediments (Madden et al., 1982) and riverside mud (Mendez et al., 1991). Little is known about the anaerobic cellulolytic bacteria from termite gut, although these insects are very efficient lignocellulose-decomposers. Recent studies (Brauman et al., submitted for publication) have indicated that their gut microbiota may provide a suitable model system to understand the electron flow involved during biopolymer degradation by anaerobic microbial communities.

In this paper, we report on a new cellulolytic *Clostridium* species, strain CT1112, isolated from the gut of a wood-feeding termite, *Nasutitermes lujiae*, from the Mayombe tropical rainforest, Congo, Central Africa.

**Materials and Methods**

**Culture methods and media.** Hungate's anaerobic techniques (Hungate, 1950; Hungate, 1969; Macy et al., 1972) were used throughout these experiments. Strain CT1112 was cultured on the mineral medium described by Balch et al. (1979) containing per liter: K₂HPO₄, 0.3 g; KH₂PO₄, 0.3 g; (NH₄)₂SO₄, 0.3 g; NaCl 0.6 g; MgSO₄·7H₂O, 0.13 g; CaCl₂, 2H₂O, 8 mg; cystein- HCl, 0.3 g; resazurin 0.2%, 1 ml; trace element solution (Imhoff-Stückle and Pfennig, 1983), 1 ml; NH₄Cl, 1 g. After autoclaving at 110°C for 40 min, the medium was cooled under a stream of O₂-free N₂-CO₂ (80–20%). NaHCO₃, 4.5 g/l, Na₂S, 0.4 g/l and carbon source were added from separately sterilized anoxic solutions; filter-sterilized growth factor solutions (yeast extract, Biotrycase, vitamins) were added when required. The media were finally adjusted to pH 7.0 and distributed into Hungate tubes, serum bottles or roll tubes containing 1.8 % agar. The cellulose used was cellulose MN 300, 20 µm (SERVA, Heidelberg, FRG). Growth was measured in Hungate tubes at 580 nm. All characterization tests were carried out in at least duplicate cultures.
Isolation procedure. Anaerobically dissected and ground guts from living wood-feeding termites were incubated on enriched liquid medium containing cellulose or directly on solid medium in roll tubes. Colonies in roll tubes showing clear zones of digested cellulose were serially diluted onto cellulose-agar in roll tubes until the pure culture was obtained. Purity was confirmed by subculturing the isolate on enriched liquid medium supplemented with 1 g/l yeast extract and Biotryptcas (Biomérieux, Crappone, France). Strain CT1112 isolated from Nasutitermes lujae was then routinely cultured both on 5g/l cellulose MN 300 and 2 g/l cellobiose.

Analytical techniques. All biochemical and physiological tests were carried out from subcultures on cellobiose minimum medium, without any growth factor. The degradation of cellulose and reducing sugars was measured using the phenol-sulfuric acid method (Dubois et al., 1956) and the ferrocyanide method (Park and Johnson, 1949), respectively. Volatile fatty acids and alcohols were analysed as previously described (Cord-Ruwisch et al., 1986). Hydrogen was measured using a gas chromatographic method with a thermal conductivity detector, with a 1.80 m Carbosphere (60-80 mesh) column operated at 85°C; gas sampling was performed with a gastight pressure lock syringe.

DNA base composition. The mol percent guanine plus cytosine of the DNA was determined at the DSM, Braunschweig, FRG. After disruption with a French pressure cell, the DNA was isolated by means of HAP chromatography (Cashon et al., 1977) and determined by HPLC using the method described by Meshbah et al. (1989).

Electron microscopy. Negative staining was performed with uranyl acetate (4% w/v in distilled water). Cells were fixed for 1 h in sodium cacodylate buffer (0.07 M, pH 7.3) containing glutaraldehyde (1.2% w/v) and ruthenium red (0.05% w/v). After washing in cacodylate buffer with ruthenium red, the samples were postfixed in OsO₄ (1% w/v in cacodylate buffer 0.07 M). Embedding was performed in Epon and ultrathin sections were stained with uranyl acetate (2% w/v in ethanol 50% w/v) and then with lead citrate. Micrographs were taken on a JEOL 1200 CX electron microscope.

Results

Isolation

The enrichment and isolation procedures yielded three anaerobic cellulolytic isolates from which strain CT1112 was selected for identification.

Colony morphology

Colonies developed to about 1 mm in diameter in 7 days, when grown on cellulose-agar at 37°C. The colonies inside the agar were circular and punctiform in shape and slightly yellow. Clearing cellulolysis zones reached up to 5–6 mm in diameter with prolonged incubation. The surface colonies spread rapidly and were diffuse.

Cellular morphology

The cells of strain CT1112 were straight to slightly curved rods about 0.5 μm wide by 4–6 μm long (Fig. 1a). The oval spores were terminal. The mature spores were 0.7 μm in width and 1.2 μm in length and caused a marked swelling of the cells (Fig. 1a). Endospores were still viable.

![Fig. 1. Morphology of strain CT 1112. a, phase contrast micrograph; b, transmission electron micrograph, phosphotungstate negative staining; c, transmission electron micrograph, thin section.](image-url)
after heating at 100°C for 10 min. Strain CT1112 was motile by peritrichous flagella (Fig. 1b) and stained Gram positive; the transmission electron micrograph of vegetative cells and showed a Gram positive cell wall profile with a thick single layer and an S-layer (Fig. 1c). Chromatographic separation of the amino acids showed that the cell wall contains m-DAP (Kandler, personal communication).

**Nutrition and growth conditions**

No yeast extract, Biotrypcase, or added vitamins were required for growth, but their presence enhanced the growth. The optimum temperature for growth was 37°C, and growth occurred in a temperature range of 20 to 48°C (Fig. 2). The optimum pH for growth was 7.5, and the pH range was 5.0 to 8.2 (Fig. 3).

**Metabolic characteristics**

Strain CT1112 is an obligate anaerobe that ferments cellulose, cellobiose, glucose, fructose, galactose, lactose, mannose, ribose, sorbose, xylose, maltose, melibiose, mannitol, sorbitol and poorly glycerol. It does not ferment arabinose, rhamnose, salicine, sucrose, trehalose, melezitose, raffinose, adonitol, dulcitol, pectin, starch, glycogen or gelatin. H₂, CO₂, acetate and ethanol are fermentation products. A doubling time of 6 h was obtained on 6 g/l cellobiose (Fig. 4).

Mol% G+C. The mol% G+C of strain CT 1112 was 39.2 ± 0.1.
### Table 1. Differentiation of strain CT1112 from other mesophilic cellulolytic Clostridia

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<th>C. chortates- bideum</th>
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+, positive; -, negative (f), uncertain; W, weak; ND, not determined; V, variable; peritr., peritrichous flagella; spher., spherical; subterm., subterminal.
Discussion

Cellulolysis occurs in the lower termites through symbiotic associations between protozoa and endo- or ectosymbionts (Brenninkmeijer, 1982; O’Brien and Slaytor, 1982). In some higher termites, cellulolysis is known to be linked to the activity of gut microflora such as the actinomycetes of the genera Streptomyces and Micromonospora (Pasti and Belli, 1985), which need aerobic conditions to develop. Cellulolysis can be also linked to enzymatic activities in the insects’ salivary glands (Veivers et al., 1982) or in posterior parts of their gut (Roulard et al., 1988).

The cellulolytic strain CT1112 has been isolated from the gut of a wood-feeding higher termite able to assimilate the main part of wood constituents such as cellulose, hemicellulose and lignin (Seifert and Becker, 1963; Noirot and Noirot-Timothée, 1969). As cellulolysis is an important source of nutrient for wood-feeding termites, selective enrichments of anaerobic cellulolytic bacteria from the gut of such termites may have been successful.

Several strictly anaerobic bacterial strains have been isolated recently from the gut of termites, such as the homoacetogens Sporomusa termittida (Breznack et al., 1988) and Clostridium mayombii (Kane et al., 1991) or sulfate reducers (Brauman et al., 1990; Trinkler et al., 1990).

Seventeen species of the cellulolytic genus Clostridium have been described up to now, four of which are thermophilic, namely Clostridium thermocellum (Ng. et al., 1977), Clostridium stercoreum (Madden, 1983), Clostridium thermopropionicum (Mendez et al., 1991) and Clostridium cellulosi (Yangling et al., 1991). Strain CT1112 can therefore be compared with the thirteen mesophilic Clostridium species described in the literature. Table 1 gives the main characteristics of these species as compared to strain CT1112.

Strain CT1112 is a Gram-positive slightly curved rod, motile by peritrichous flagella and forms swollen oval terminal spores. Strain CT1112 differs greatly from strain CT1112 (DSM 5398), Clostridium celluloferrantis (Vanlig et al., 1991) and Clostridium lactoceillum (Murray et al., 1986) use raffinose, salicin, sucrose and trehalose but not sorbitol; they have a lower G+C% content of DNA (34–36) than that of strain CT1112 (39).

According to our results, strain CT1112 is a new mesophilic cellulolytic bacterium using sorbitol, mannitol and lactose but not arabinose and pectin, and producing mainly acetate, ethanol, H2 and CO2. We propose that strain CT1112 be placed in the genus Clostridium as a new species, Clostridium termidisi sp. nov.

Description of Clostridium termidisi sp. nov.: termitidis. L.n. tarmes, termitt- (L.L. var. terms, termitt-) worm that eats wood; M.L. adj. termidisi pertaining to the termite.

Cells are straight to slightly curved Gram-positive rods, 0.5 μm by 4–6 μm, peritrichously flagellated. Oval terminal spores cause a marked swelling of the cells.

The deep colonies which develop in cellulose-agar roll tubes are small, circular and slightly yellow. The surface colonies are widespread on agar.

Obligate anaerobe. Ferments cellulose, cellobiose, glucose, fructose, galactose, lactose, mannose, ribose, sorbose, xylose, maltose, melibiose, mannitol, sorbitol and poorly glycerol. Acetate, ethanol, H2 and CO2 are fermentation products.

The temperature optimum for growth is 37°C. The range is 20 to 48°C. The pH optimum for growth is 7.5. The range is 5 to 8.2. Growth factors are not required, but yeast extract, biotin, or vitamins enhanced growth.

The mol% G+C is 39.2±0.1.

Isolated from the guts of the wood-feeding termite Nasutitermes lujae, from the Mayombe tropical rainforest, Congo, Central Africa.

The type strain is strain CT1112 (DSM 5398).

Acknowledgements. We are indebted to K. D. Jahnke (DSM) for determining the DNA base ratio, O. Kandler for determining the cell wall content of m-DAP, C. Allassia for preparing the electron micrographs, B. Olliver for helpful discussion, and J. Blanc for revising the English.

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