Supplementary Methods

(a) *Termites*

Nasutitermes takasagoensis was collected and maintained as described previously (Tokuda *et al.* 1997). *N. walkeri* was provided by Dr Nathan Lo at the University of Sydney. Mature worker-caste termites were used in the present study unless otherwise indicated.

(b) *Preparation of crude and pellet extracts*

Five termites were used for the determination of cellulase activity. The midguts or the enlarged part of the hindguts (P2–P5; Bignell 1994), hereafter called 'the hindgut', were collected in 100 μl of proteinase inhibitor solution (Complete Mini EDTA-free; Roche, Mannheim, Germany) prepared with distilled water. Each sample was sonicated at 360 W cm^{-2} (0.5 s × 6 times) at 4°C and centrifuged at 20 630*g* for 20 min. Supernatants recovered are referred to as crude extracts. Each pellet was suspended with 100 μl of the same solution and centrifuged at 20 630*g* for 20 min. This step was repeated three times to eliminate water-soluble proteins. Then, each pellet was suspended with 100 μl of detergent reagent (CelLytic^{TM} B Cell Lysis Reagent; Sigma-Aldrich, St Louis, MO, USA) and vortexed vigorously for 10 s. The suspensions were incubated on ice for 10 min and centrifuged at 20 630*g* for 20 min. The supernatants are referred to as pellet extracts.

(c) *Detection of cellulase activity on SDS– and native-PAGE gels*

Zymogram analyses were performed as follows; 5 μl of crude or pellet extract (except for midgut crude extract, which was diluted 10 times with distilled water) was mixed with 10 μl of Laemmli's sample buffer (Bio-Rad, Hercules, CA, USA) for SDS–PAGE or 10 μl of native sample buffer (Bio-Rad) for native-PAGE. The samples were immediately applied onto 10% polyacrylamide gel

containing 0.1% (w/v) carboxymethyl cellulose (Sigma-Aldrich) and run with or without SDS at 20 mA at 4°C. After the electrophoresis, the gels were incubated at room temperature with 100 ml of McIlvaine's citrate–phosphate buffer (McIlvaine 1921) at pH 6.5 for 15 min. The buffer was replaced with 0.1% (w/v) Congo red in distilled water and the gels were stained for 10 min at room temperature. Then, the gels were destained with 1 M NaCl, which removed excess dye and formed white halos by cellulolytic activity in the gel.

(d) *Crystalline cellulose degrading activity*

Crude or pellet extract (30 μl) was incubated with 200 μl of 2% microcrystalline cellulose (Sigmacell Type 20; Sigma-Aldrich) in McIlvaine's buffer at 37°C for 1 h. Because the hindgut pellet showed the maximal cellulase activity at pH 6.5 (see Supplementary Figure S1), cellulase activity was measured at pH 6.5 for the hindgut extracts. On the other hand, because the optimal pH of endo-β-1,4-glucanase produced in the midgut is 5.8 (Tokuda *et al.* 1997), the midgut cellulase activity was measured at pH 5.8. During incubation, tubes containing the enzyme reaction were shaken intensively (1200 oscillations per minute) to suspend the substrate. The reaction was terminated on ice, and tubes were briefly centrifuged to collect supernatants. To detect reducing sugars (consisting of short cello-oligosaccharides) released into the supernatants, each supernatant (115 μl) was mixed with 1 ml of tetrazolium blue reagent (Jue & Lipke 1985) and boiled for 5 min. Photometry was performed as previously described (Tokuda *et al.* 1997) and the amount of reducing sugars was expressed as glucose equivalents.

(e) *Definition of enzyme unit*

One unit (U) of cellulase activity is defined as the amount of enzyme that produces 1 μmol of reducing sugar (glucose equivalents) per minute.

(f) *Antibiotic treatment*

Termites (six workers plus one soldier) were placed in a Petri dish containing a 7 cm × 5 cm filter paper soaked in 1 ml of either 5.4 mM (i.e. 2 mg ml^{-1} in sterilized distilled water) ampicillin or sterilized distilled water. Five replicates of each plate were maintained at room temperature. The filter papers treated with antibiotics or distilled water were replaced every two days. After one week, termites were dissected and cellulase activity was measured as described above. The same experiments were performed for *N. takasagoensis* and *N. walkeri*. Although 52 of 60 *N. takasagoensis* workers survived during the experiment, 56 of 60 *N. walkeri* workers died during the feeding experiment. Therefore, only cellulase activity of *N. takasagoensis* was measured.

Reference in addition to those listed in the text:

Jue, C. K. & Lipke, P. N. 1985. Determination of reducing sugars in the nanomole range with tetrazolium blue. *J. Biochem. Biophys. Meth.* **11**, 109-115.

McIlvaine, T. C. 1921 A buffer solution for colorimetric comparison. *J. Biol. Chem.* **49**, 183–186.