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New developments towards the understanding of the *Clostridium thermocellum* cellulosome

Summary

A list of 71 readings frames for potential cellulosomal components was derived from the genomic sequence of *C. thermocellum*. A careful annotation of the cellulosomal ORFs including the putative module architecture is presented. The 13 major components of the cellulosomes were separated by denaturing 2D-gels and identified with proteomic methods (MALDI-TOF). Three of the new components were newly identified and furtheron cloned and biochemically characterized: an endo-ß-xylanase Xyn10D, an endo-xyloglucanase Xgh74A producing hepta- and octomers from Tamarind xyloglucan, and Cel9R, a new type of processive endo-ß-1,4-glucanase producing cellotetraose from amorphic and crystalline cellulose.

The role of cellotetraose in the catabolism of natural cellulose by *C. thermocellum* is briefly discussed.

C. thermocellum has a highly efficient cellulase system

Filter paper is degraded by a growing culture of *C. thermocellum* within 2 days at 55 to 68 °C (Fig. 1). Fermentation products are ethanol, acetate, CO_2 and hydrogen. The bacterial cells adsorb tightly to the substrate surface and take up the hydrolysis products directly (Fig. 2). *C. thermocellum* forms small colonies on anaerobically prepared agar plates. Colonies hydrolyze crystalline cellulose arond the colonies on the plate surface (Fig. 3).

Fig. 1: Hydrolysis of filter paper strips by a *C. thermocellum* culture. The insert after 24 hrs of incubation shows the dissolving paper fibres.

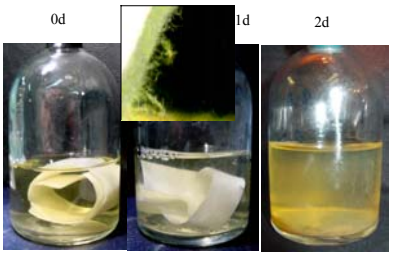
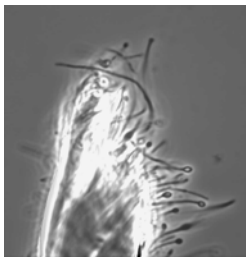


Fig. 2: *C. thermocellum* cells attached to a cellulose fibre (phase contrast micro-scopy 1000 x)



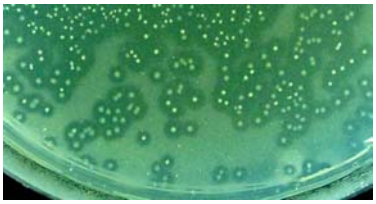
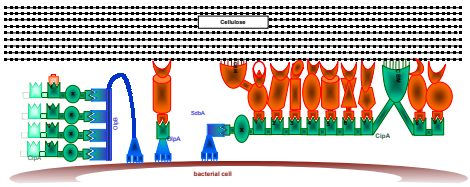


Fig. 3: *C. thermocellum* colonies on agar plates (anaerobic). Cellulose on the plate surface (turbid) is hydrolyzed around colonies

The cellulosome is an extracellular multienzyme complex

The efficiency of the plant fibre hydrolysis by *C. thermocellum* is due to the formation of an extracellular multienzyme complex which includes all enzyme components necessary for hemicellulose (xylan, xyloglucan, glucan), pectin and cellulose degradation (Fig. 4). The components (in **red**) are fixed by a matrix protein, the scaffoldin CipA (in **green**), which again is bound to the cell wall by other structural proteins (in **blue**). The scaffoldin and many of the components have substrate binding modules (CBM).

Fig. 4: Cellulosome structure: The scaffoldin (green) connects the cells with the enzyme components and has a binding module for binding to the surface of the substrate.



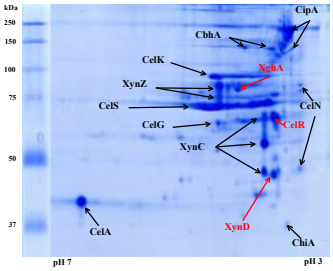
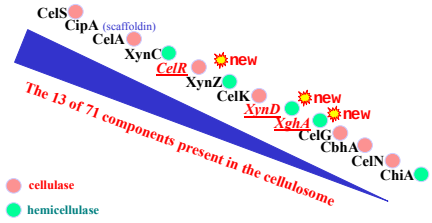


Fig. 5: *C. thermocellum* cellulosomal proteins separated by IEF and urea-PAGE. New components in **red**

Cellulosome composition

Cellulosomes were prepared from cellulose grown cells and the components were separated by 2D-gel electrophoresis (Fig. 5). The 13 major components were identified with proteomic methods (MALDI-TOF). Three of them were newly identified, cloned from genomic DNA and biochemically characterized: endo-ß-xylanase **Xyn10D**, endo-xyloglucanase **Xgh74A**, and endo-glucanase **Cel9R**. The amount of the proteins was determined (Fig. 6).

Fig. 6: Cellulosomal components of *C. thermocellum*, grown on cellulose: quantitative analysis of the 13 major components. The newly detected components are marked in **red**.



New cellulosomal components

Cel9R: the most abundant GHF9 enzyme in the cellulosome is a processive endoglucanase splitting cellotetraose residues from the non-reducing end of cellulose molecules, which are only slowly further processed. See Fig. 7.

Xyn10D: belongs like all other xylanases in the cellulosome to family GHF10. Endoxylanase, splitting xylans through xylo-oligosaccharides to xylobiose + less xylose and xylotriose.

Xgh74A: is hydrolyzing the xyloglucan backbone at the reducing end of underivatized glucosyl residues to hexa- to nona-saccharides (Fig. 8). Endo-xyloglucanase of GHF74.

Fig. 7: Time course of digestion of phosphoric acid swollen cellulose by different cellulases. G1=glucose, G2=cellobiose etc.

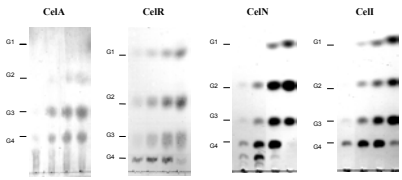
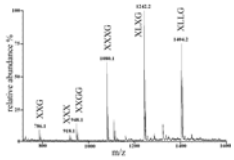


Fig. 8: ESI-MS of the hydrolysis products from tamarind xyloglucan by Xgh74A (thanks to Schmitt-Kopplin).



Conclusion:

1. A great number of potential genes (71) code for cellulosomal enzymes, but only 13 of them are major components in the protein complex.
2. Of the major components 7 are cellulases and 5 are hemi-cellulases (3 of them GHF10 xylanases). Hemicellulolysis thus plays an important role in the break-down of plant biomass, even though *C. thermocellum* cannot utilize the pentose sugars produced.
3. At least 3 of the cellulosomal cellulases form cellotetraose as the major initial hydrolytic product, which could be via phosphorolysis an energy-efficient substrate for *C. thermocellum (*no extra ATP needed for transport and substrate phosphorylation). Further break-down is slow.
4. The hydrolysis of plant fiber is very complex and needs a great number of different enzymes.