3D Electron Tomography of Switchgrass Cell Wall Deconstruction by *Clostridium cellulolyticum*

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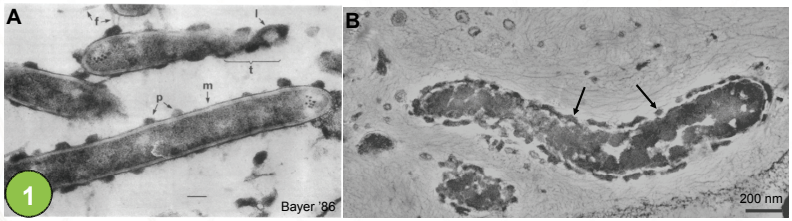
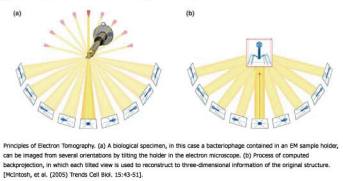
Abstract

Among the many biomass-digesting microorganisms, a number produce structured biomass-degrading enzyme complexes. These complexes, called cellulosomes, are known to contain a variety of biomass-degrading enzymes docked to structural proteins termed "scaffoldins," which also often contain carbohydrate binding domains. Cellulosomes and their structural and enzymatic components may play important roles in bioenergy production and in future biotechnologies. Several cellulolytic members of the genus *Clostridium* exhibit cellulosomes. In order to understand the biomass-degrading properties of these organisms and the structure and organization of cellulosomes, we have employed electron tomography of high-pressure frozen/freeze substituted *C. cellulolyticum* cultures grown on native switchgrass to examine the complex 3D ultra-structure of the whole, intact cell wall degrading system at 3-5 nm resolution. We have observed cell wall deconstruction mechanisms like burrowing and clearing of localized zones of cell walls that differ from the mode of action of fungal free-enzyme digestion. Electron tomography has also revealed structural details of the tethers that anchor cellulosomes to bacterial cells.



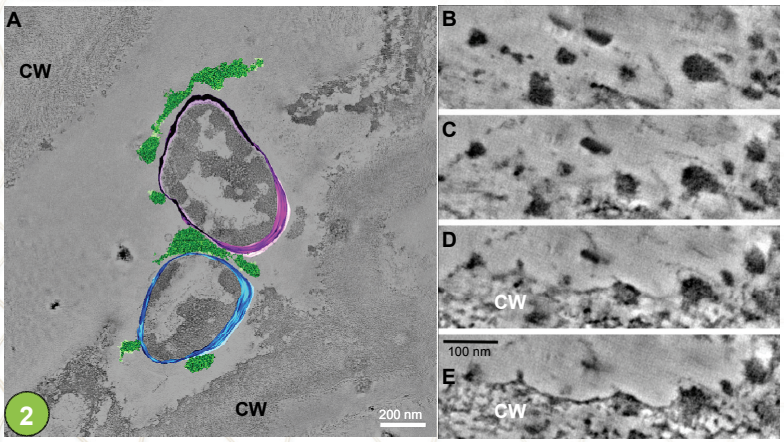
TEM micrographs of CF-labeled cellobiose-grown cells of *C. thermocellum* from the classic study by Bayer and Lamed (A). Tomographic slice of a switchgrass-grown *C. cellulolyticum* from this study processed by high-pressure freezing and freeze substitution.



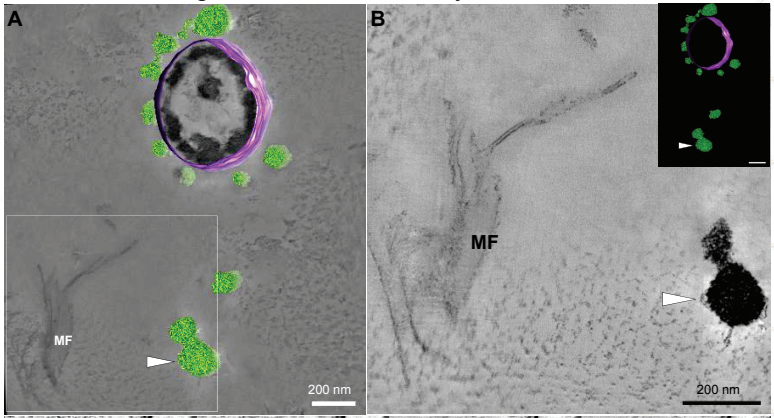
Principles of Electron Tomography. (a) A biological specimen, in this case a bacteriophage contained in an EM sample holder, can be imaged from several orientations by tilting the holder in the electron microscope. (b) Process of computed backprojection, in which each tilted view is used to reconstruct to three-dimensional information of the original structure. [McIntosh, et al. (2005) Trends Cell Biol. 15:43-51].

Methods

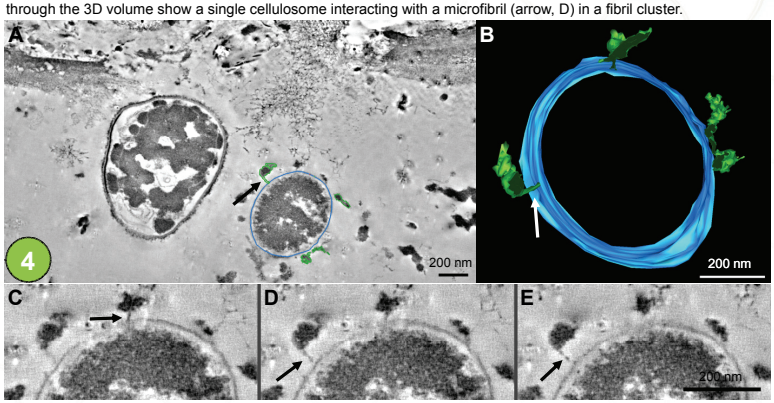
- *C. cellulolyticum* (ATCC 35319) were grown in GS medium with cellobiose for 2-3 days at 37ºC; sterilized, ground, untreated switchgrass added at 37ºC for 2 additional days.
- Samples were cryo-preserved by high-pressure freezing in a Baltec HM100, freeze substitution and low temperature HM-20 embedding in a Leica AFS, and sectioned to 60 or 200 nm on an Leica UCT ultramicrotome. Sections were stained w/ $KMnO_4$ for 6 min and Uranyl Acetate for 6 min.
- Dual-axis tilt series were captured using Serial EM on an FEI Tecnai G2 Twin 200 kV LaB6 TEM with a 2K Gatan Ultrascan CCD camera at 0.54 or 1.08 nm pixel size. Tomograms were assembled using IMOD, and modeled using the IMOD and Chimera software packages.



From tomograms of *Clostridium cellulolyticum* digestion of switchgrass. (A) Tomogram slice with superimposed segmentation and isosurface model. Bacteria (pink and blue membrane surfaces) "burrow" into cell walls by creating a pocket of localized digestion. Massive amounts of cellulosomal protein (green) stud the surface of each bacterium. Polycellulosomes (A, upper left; B-E) interact with the surface of switchgrass cell walls. In B-E, serial slices with image spacing of ~8 nm show free cellulomes burrowing into a scalloped cell wall surface.



Tomogram slice images and electron density isosurfaces of *C. cellulolyticum* cells and cellulosomes interacting with cell wall fragments. Cellulosome-covered cells and free polycellulosomes are often found near highly fibrillated cell wall fragments that display both filament and thin-sheet morphology (A, B). C-F are serial slices taken every ~4 nm through the 3D volume show a single cellulosome interacting with a microfibril (arrow, D) in a fibril cluster.



Tomogram slices and surface rendered segmentation of bacterial cells and tethered cellulosomes. C-D are serial slices taken every ~8 nm through tethered cellulosomes. These tethers are seen at one end of most polycellulosmes found near the bacterial cell surface and are approximately 5 nm in diameter and up to 50 nm in length.

Conclusions

- Electron tomography of cryo-preserved *C. cellulolyticum* growing on switchgrass provides 3D ultra-structural data of the entire, active, complex system *in situ* at 3-5 nm resolution.
- *C. cellulolyticum* interacts with switchgrass cell walls by burrowing into a local region, unlike free cellulases.
- 3D ultrastructure analysis reveals variable structure depending on substrate interaction and cell attachment.

Future Directions

- Refined 3D electron density maps of cellulosomes using hybrid techniques that incorporate X-ray crystallographic and molecular modeling data to contibute to a mechanistic model for cellulosomes.
- Use immuno-EM to localize specific cellulosome proteins within tomograms.
- Investigate the structure and impact of mutant and synthetic cellulosomes.

References

Bayer EA, R Lamed (1986). Ultrastructure of the Cell Surface Cellulosome of Clostridium thermocellum and Its Interaction with Cellulose. J. of Bacteriology, 167(3): 828-836.

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