The hyper thermophilic cellulase CelA from *C. bescii* is indifferent to the level of crystallinity in biomass

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Background

- Biomass recalcitrance and cellulose crystallinity remain the main impediments to cost effective conversion of biomass to fermentable sugars.
- *C. bescii*, a hyperthermophilic lignocellulose degrading anaerobe, is an efficient cellulolytic microbe and one that offers potential for consolidated bioprocessing.
- *C. bescii* uses multi-catalytic domain glycoside hydrolases to efficiently degrade biomass. The most prevalent cellulase in its exoproteome, CelA, is highly active at temperatures reaching 90°C and is the most efficient single gene product ever tested. Additionally, it exhibits a new cavity forming deconstruction mechanism different from that of fungal cellulases found in most commercial preparations.

Approach

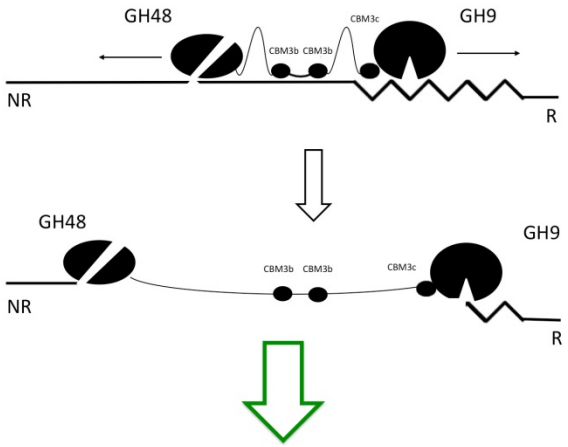
- Biomass samples with different levels of crystallinity and composition were prepared using known pretreatment techniques to control the crystallinity content and selectively remove different biomass components.
- Deconstruction of these biomass samples by CelA was followed using sugar release assays, advanced imaging, immunolabeling, kinetic modeling, and binding analyses.

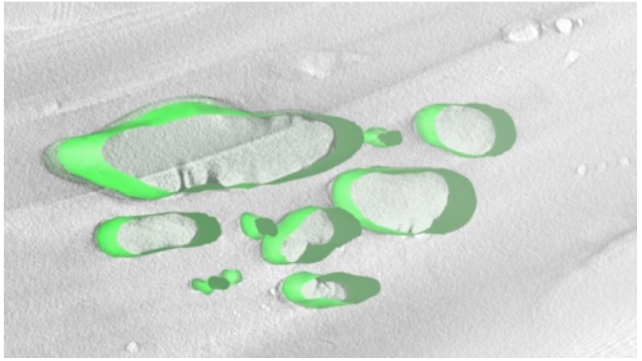
Outcome

- CelA was shown to break down cellulose with high crystallinity to the same extent as low crystallinity cellulose – a characteristic never reported for other cellulases.
- CelA exhibits a cavity forming mechanism on all types of biomass tested.
- CelA struggles when deconstructing biomass rich in lignin and thus we are pursuing engineering efforts to improve this enzyme for even better performance on non pretreated biomass.

Significance

This discovery demonstrates that nature's strategies for biomass conversion are diverse and that enzymes with new characteristics can still be discovered and isolated for improving biomass deconstruction applications.





Schematic and TEM micrograph of the cellulose deconstruction mechanism of CelA. The GH48 and GH9 catalytic domains work in opposite directions. As long as the CBMs are actively bound to the substrate, CelA will produce cavities because the length of the linker peptides limits the separation of the two catalytic domains.

Brunecky, *et al.,* "The Multi Domain *Caldicellulosiruptor bescii* CelA Cellulase Excels at the Hydrolysis of Crystalline Cellulose," *Scientific Reports* 7:9622 (September 2017) | DOI:10.1038/s41598-017-0895-w

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