Biomass deconstruction by *C. thermocellum*-mediated dynamic redistribution of cellulosomes

Background/Objective

- *C. thermocellum* efficiently deconstructs plant biomass into fermentable sugars using surface-associated enzyme complexes called cellulosomes. We hypothesize that the organization of the enzyme–microbe–substrate (EMS) region, and its dynamic reorganization during growth, drives high cellulolytic activity. We use super-resolution microscopy with quantitative clustering algorithms to map and quantify cellulosome localization during growth on Avicel, a model cellulosic substrate.

Approach

- A dual-labeling strategy was implemented to visualize *C. thermocellum* cellulosomes, combining immunofluorescent probes and photoactivatable GFP fusion proteins targeting two different regions of the cellulosomes, to acquire single-molecule localizations by PALM/STORM microscopy.
- The nanoscale spatial organization of cellulosomes was quantified by applying unsupervised density-based clustering to compare cellulosome enrichment patterns across growth conditions.

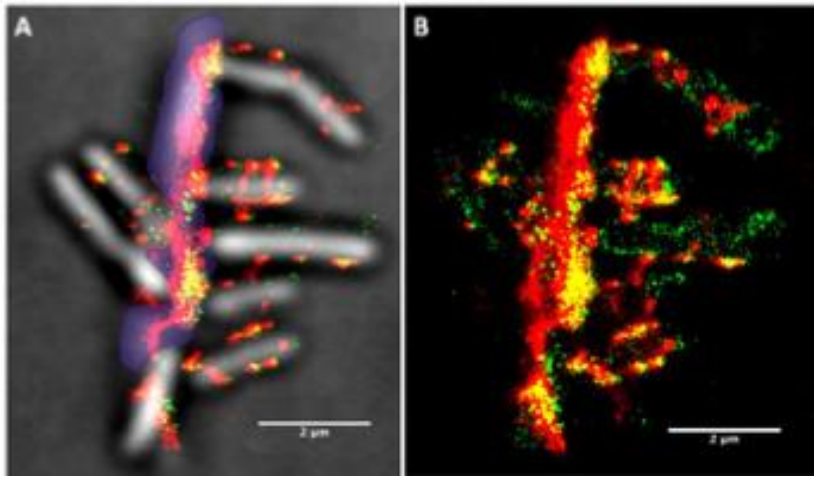
Results

- Free *C. thermocellum* cells display large cellulosome clusters in log phase that become smaller during growth, especially in stationary phase. When bound to Avicel, the local cellulosome concentration at the EMS interface was high and actively increases in substrate-bound cells relocating their cellulosomes to the contact point.

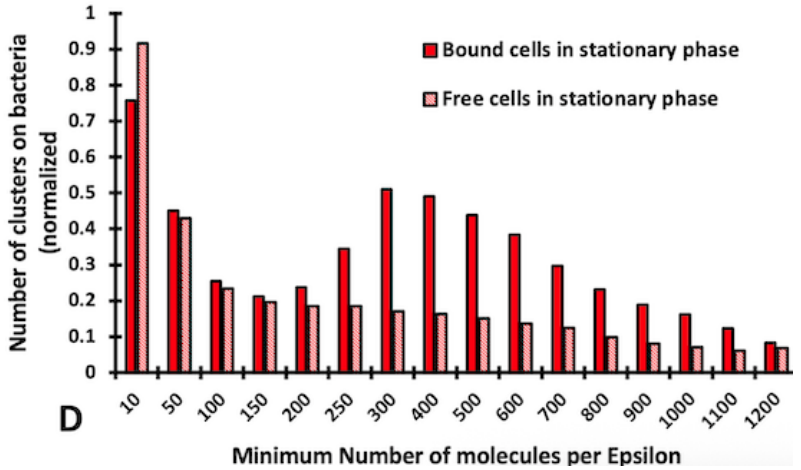
Significance/Impacts

- Improved understanding of this deconstruction mechanism by *C. thermocellum* is aiding engineering efforts for more efficient biomass deconstruction approaches for consolidated bioprocessing.
- The imaging approaches adapted here for thermophiles are now being utilized to study coculture dynamics and fitness, understand synergy and competition by microbes and their enzymes, and are ultimately being leveraged to design better cocultures and coculture partners.

Yarbrough, J.M., et al., Life Science Alliance, 2026. **9**(3). DOI: 10.26508/lsa.202503239



Stationary-phase images of *C. thermocellum* grown on Avicel: (A) white-light image overlaid with fluorescence and (B) fluorescence alone. The Avicel particle is shaded purple in (A), exhibiting elevated fluorescence at the EMS region, indicating a high local concentration of cellulosomes.



Unsupervised density-based clustering of super resolution fluorescence data showing Avicel bound cells display large clusters of cellulosomes whereas free cells are depleted in cellulosomes in stationary phase.