Identification of Cellulolytic Hot Spring Organisms Through Bioorthogonal Labeling

Nicholas J. Reichart *, Chemistry and Biochemistry, Montana State University, Bozeman
Zackary Jay, Chemistry and Biochemistry, Montana State University, Bozeman
Viola Krukenberg, Chemistry and Biochemistry, Montana State University, Bozeman
Rachel Spietz, Chemistry and Biochemistry, Montana State University, Bozeman
Roland Hatzenpichler, Chemistry and Biochemistry, Montana State University, Bozeman

*Indicates Presenter

Bioprocessing of cellulose from plant waste into ethanol has been a focus of the renewable energy field for several decades. The recent developments of genome sequencing technologies have reinvigorated the topic. Identification of genes and the potential to degrade cellulose has been the major outcome thus far. High-throughput techniques to link taxonomy and in situ function of the organisms responsible for these processes have, however, been lacking. Bioorthogonal non-canonical amino acid tagging is a nondestructive method to fluorescently label active, protein-synthesizing cells that can later be separated from a community using fluorescent activated cell sorting (BONCAT-FACS). Biomass collected from a Yellowstone National Park hot spring was incubated with four cellulose substrates to identify organisms that were preferentially active in the presence, but not the absence of cellulose substrates. A short timeframe (2 weeks) bioorthogonal labeling incubation and a longer enrichment (6 weeks) incubation were compared for differences in community composition to highlight the competition of an incubation that is typically missed with enrichment schemes. Over 14 days of incubation the community began to shift to a simplified population comprised of Aquificae, Deinococcus-Thermus, or Chloroflexi. However, at early time points, the active community was comprised by a multitude of uncultured and cultured organisms including Fervidibacteria, Aigarchaeota, or Thermotogae which were eventually outcompeted by dominating organisms in the later time points. BONCAT-FACS outlines a novel approach of using single-cell bioorthogonal labeling to profile a microbial community's activity regarding biotechnology industry interest.