DISCOVERY, CHARACTERIZATION AND ENGINEERING OF BACTERIAL THERMOSTABLE CELLULOSE-DEGRADING ENZYMES

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Lignocellulose is the most abundant biomass on Earth, and thus our largest organic carbon reservoir. Enzymatic depolymerization of recalcitrant polysaccharides, notably cellulose, is a major cost driver in accessing the renewable energy stored within lignocellulosic biomass. Natural biodiversities may be explored to discover microbial enzymes that have evolved to conquer this task in various environments. We are studying novel enzymes from various biodiversities for the conversion of lignocellulosic materials, using (meta)genome mining and functional screening of fosmid libraries. Targeted biodiversities include deep-sea hot vents of the Arctic mid-ocean ridge (AMOR), the microbiome of the wood-eating Arctic shipworm, thermophilic enrichment cultures from biogas reactors, the Svalbard reindeer gut microbiome, and publicly available metagenomic data from various hot environments. Bioprospecting of the different biodiversities has so far resulted in the discovery of approximately 20 novel enzymes active on lignocellulosic substrates. The significant differences in the origin of the enzymes is reflected in their properties, both beneficial and challenging, and provide us with interesting engineering targets for improved performance in industrial settings.

We will present case studies, including work on a novel thermostable cellulase named mgCel6A, with good activity on sulfite-pulped Norway spruce. This enzyme consists of a glycoside hydrolase family 6 catalytic domain (GH6) connected to a family 2 carbohydrate binding module (CBM2) and both the activity profile and predicted structural similarities to known cellulases suggest that mgCel6A is an endo-acting cellulase. Comparison of the full-length enzyme with the catalytic domain showed that the CBM strongly increases substrate binding, while not affecting thermal stability. However, importantly, in reactions with higher substrate concentrations the full-length enzyme was outperformed by the catalytic domain alone, underpinning previous suggestions that CBMs may be less useful in high-consistency bioprocessing. This enzyme is currently being targeted for rational engineering in an effort to decrease the pH optimum and improve the pH stability.

Other case studies include GH48 cellulases and lytic polysaccharide monooxygenases (LPMOs). One important aspect of this work concerns the possible assembly of novel enzyme cocktails for lignocellulose processing that can compete with exiting commercial cocktails, which are primarily composed of fungal enzymes. Thus, comparative studies of our most promising bacterial enzymes with their well-known fungal counterparts are also being conducted.