Improved Enzyme Mixtures for Biomass Deconstruction

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Project Goals: To perform fundamental research on cell wall degrading enzymes that will lead to the development of more efficient mixtures for the conversion of biomass to fermentable sugars.

Currently available commercial enzymes for biomass depolymerization are complex and poorly defined mixtures of activities and proportions. Many of the enzymes, secreted by fungi such as *Trichoderma reesei*, are not necessary for industrial biomass conversion, whereas other critical enzymes are absent or present at sub-optimal proportions. Our long term goal is to construct enzyme mixtures of optimized composition. To do so, we are building synthetic mixtures starting with pure, individual enzymes. Mixtures are optimized using statistical Design of Experiment and robotic liquid handling in an integrated system called GENPLAT. The resulting mixtures are further improved by the addition of novel enzymes that are lacking from commercial preparations such as Accellerase 1000, CTek2, and Spezyme CP. To date, we have constructed optimized cocktails containing up to 15 components. Some contribute to glucose (Glc) yield, others to xylose (Xyl) yield, and some enhance yield of both sugars from various pretreated biomass materials (Banerjee et al., 2010a). The required enzymes and their optimized proportions depend on the pretreatment conditions and biomass composition (Banerjee et al., 2010b).

We are also identifying novel biomass deconstruction enzymes and are testing their contribution to Glc and Xyl yield when combined with synthetic mixtures and with commercial enzymes. Secreted α-xylosidase (AX) is predicted to be important for release of Glc and Xyl from plant cell walls, especially from dicots, which have high levels of xyloglucan. Secreted AX's are rare in nature. We identified and purifed a secreted AX from *Aspergillus niger*. AX activity and even the encoding gene are absent from *T. reesei*. AX in combination with β-glucosidase depolymerizes pea xyloglucan to free Xyl and Glc (Scott-Craig et al., 2012).

Another novel enzyme is α-fucosidase, which is predicted to be essential for the deconstruction of fucosylated xyloglucan, found in many dicot plants. As the terminal sugar on xyloglucan sidechains, fucose must be removed before the other enzymes (β-galactosidase, α-xylosidase, xyloglucanase, and β-glucosidase) can act.

The use of mixed plant species as biomass feedstock has potential economic and environmental advantages over monocultures. The "Sustainability" area of GLBRC is actively investigating mixed prairie as biomass feedstock. Mixed prairie and old fields contain a high percentage of herbaceous dicots (known as forbs). The cell wall composition of forbs is quite different from grasses, and little is known about what pretreatments and enzymes will be effective on forbs. In consultation with the Sustainability Area, our lab has developed a model set of forbs for fundamental studies on the factors that limit their digestibility and hence potential as biofuel "crops". The plants being studied are goldenrod (*Solidago canadensis*), Queen Anne's lace (*Daucus carota*), lambsquarters (*Chenopodium album*), and milkweed (*Asclepias syriaca*).

These species grow over a wide geographical range, they are taxonomically diverse, they are often very common in old fields and prairies, and although weedy, they are not noxious. We are testing pretreatments and optimized enzyme cocktails for release of Glc and Xyl from these model forbs. We hypothesize that the recalcitrance of forbs is due to some combination of the ineffectiveness of current thermochemical pretreatments, release of enzyme inhibitors by the pretreatments, or absence of certain essential enzyme activities in current commercial enzyme mixtures.

References

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