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**Fine-tuned identification of glycoside hydrolase specificity on plant cell walls**

Combining glycome profiling and nanostructure initiator mass spectroscopy to characterize cell-wall enzymatic hydrolysis

**The Science**

Glycoside hydrolases (GH) are enzymes that release sugar from cellulose, hemicellulose, and other polysaccharides. Understanding the specificity of GH enzyme reactions in the context of the plant cell wall is essential to providing more efficient ways to deconstruct plant biomass for biofuels production.

**The Impact**

This study offers a ‘real-time’ exploration of enzymatic break-down of cell walls and provides not only a characterization of substrate preferences for individual enzymes or enzyme mixtures, but also reveals the product profile generated from the reaction. Glycome profiling can be used to identify biomass pretreatment regimes that effectively contribute to efficient enzymatic deconstruction of complex hemicellulose/cellulose infrastructure, and illuminates the specific carbohydrate moieties that are targeted by the enzyme or enzyme mixture. Furthermore, oxime-NIMS provides a quantitative assessment of the oligosaccharide products generated, which not only sheds light on the mechanism of action of the enzyme, but can indicate what products are available for downstream application, or conversely, the levels of inhibitory products that can reduce enzyme efficiency.

**Summary**

Glycome profiling and oxime-NIMS were used to explore the efficacy of three *Ruminiclostridium thermocellum* enzymes in deconstructing non-pretreated and ammonia fiber expansion (AFEX)-pretreated corn stover and switchgrass. These enzymes fall into three classes of glycosyl hydrolases: CMX00\_3a is from GH5 subclass 4, which contains numerous multifunctional enzymes capable of utilizing glucan, xylan, and/or mannan-based substrates; XynY, a GH10 xylanase; and XynA, a GH11 xylanase. The results showed that the GH5 and GH11 enzymes could break down both hexose- and xylose-backbone substrates and thus had a broader specificity, whereas GH10 was highly effective in breaking down a wide range of xylan-based substrates, but was not effective on glucan-based substrates. Furthermore, glycome profiling demonstrated a clear improvement in hydrolysis when biomass was AFEX-pretreated before hydrolysis, due to improved steric accessibility of substrate to the enzymes. These methods offer fine-tuned, quantitative analysis of enzyme specificities and can help functionally annotate carbohydrate-active enzyme databases that are organized according to genomic data.

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**Publications**

Walker JA, Pattathil S, Bergeman LF, Beebe ET, Deng K, Mirzai M, Northen TR, Hahn MG, Fox BG “Determination of Glycoside Hydrolase Specificities During Hydrolysis of Plant Cell Walls Using Glycome Profiling”. Biotechnol. Biofuels. 10 (31), (2017) [DOI: 10.1186/s13068-017-0703-6]

**Related Links**

<http://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-017-0703-6>

**PM Recommendation for SC Web Publication**

[Yes or No]