Enzyme tools and carbohydrate tools for bioproducts development

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Biomaterials

Levulinic acids

Heat and power

Formic acids

Bioethanol

Deconstruction or combustion

Enzymatic modification

Biomaterials
Xylans represent abundant renewable resources for the development of bioproducts.
GH115 enzymes are the only known enzymes that target GlcA/MeGlcA from high molecular-weight xylans.

Sidegroup chemistry

Prebiotic activity, rheology, solubility, material attributes
In nature, carbohydrate-active enzymes are produced in organisms from various habitats including harsh environmental conditions.
Select bacteria from unique habitats

Salt marsh cord grass in the Chesapeake Bay

Marine bacterium
Saccharophagus degradans

Composts of manure with grass and rice straw in Japan

Alkaliphilic bacterium
Amphibacillus xylanus

Tolerance in high salt condition?

Tolerance in alkaline condition?
Produce enzymes using biotechnology

Source organism

ATCGCTAGTACG
GCATGCACTGTG
CATAATTCCAGTA
CGTTTGGGATCG

Obtain gene of interest through genomic DNA or direct synthesis

Amplification

Insert into plasmid

Transform E. coli cells

Genetically modified E. coli cells

Induce protein expression

Enzyme purification

Bacterial culture
Both enzymes demonstrated activity towards glucuronoxylans and oligomers with preference towards internally substituted residues.

\[ k_{\text{cat}} = 29.2 \pm 1.6 \text{ s}^{-1} \]
\[ k_{\text{cat}} / K_m = 5.8 \text{ s}^{-1} \text{mM}^{-1} \]

\[ k_{\text{cat(app)}} = 535.8 \pm 25.2 \text{ s}^{-1} \]
\[ k_{\text{cat(app)}} / K'_m(app) = 10.1 \text{ s}^{-1} \text{mM}^{-1} \]

\[ K_m = 5.0 \pm 1.0 \text{ mM} \]
\[ K'_m(app) = 53.2 \pm 3.9 \text{ mM} \]
\[ k_{\text{cat(app)}} / K'_m(app) = 8.8 \text{ s}^{-1} \text{mM}^{-1} \]
AxyAgu115A demonstrated better performance in alkaline condition

AxyAgu115A performance was higher than SdeAgu115A, particularly at pH values above 9.0.

Increase in substrate solubility in alkaline condition increased substrate accessibility.


Grey bars, beechwood glucuronoxylan

White bars, Oat spelt xylan
Consistent with the marine origin of SdeAgu115A, salt activation of enzyme activity was observed for SdeAgu115A but not AxyAgu115A.
AxyAgu115A displayed higher activity towards complex xylans compared to SdeAgu115A

<table>
<thead>
<tr>
<th>Polymeric substrates</th>
<th>Activity (μmol product/min/μmol enzyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SdeAgu115A</td>
</tr>
<tr>
<td>Beechwood glucuronoxylan</td>
<td>2470 ± 70</td>
</tr>
<tr>
<td>Spruce arabinoglucuronoxylan</td>
<td>917 ± 6</td>
</tr>
<tr>
<td>Oat spelt glucuronoarabinoxylan</td>
<td>24 ± 1</td>
</tr>
</tbody>
</table>

Accommodation of complex xylans was consistent with docking analysis that predicted accessibility of AxyAgu115A to branched xylo-oligosaccharides.
Thin layer chromatography

Scanning transmission X-ray microscopy

LC-MS/MS

Colorimetric analysis

Enzyme coupling method

Glucuronoxylan + H₂O \xrightarrow{\text{GH115}} Xylan + GlcA

Uronate dehydrogenase

GlcA + NAD⁺ + H₂O \xrightarrow{\text{Gluconate dehydrogenase}} Glucarate + NADH + H⁺

NADH is measured by the increase in absorbance at 340 nm.

Library/pierce-protein-methods/overview-mass-spectrometry.html

http://unicorn.mcmaster.ca/research/projects/intro/polySTXMintro-all.html

https://www.spectrumchemical.com
# Pros and cons of existing techniques

<table>
<thead>
<tr>
<th>Separation techniques</th>
<th>Pros</th>
<th>Cons</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size exclusion</td>
<td>Identified analytes above 9 kDa</td>
<td>Compounds with the same size co-elute</td>
<td>1</td>
</tr>
<tr>
<td>Ion chromatography</td>
<td>Separated neutral and acidic oligomers</td>
<td>Substitution position of MeGlcA was not confirmed</td>
<td>2</td>
</tr>
<tr>
<td>HPAEC-PAD</td>
<td>Effective separation of oligomers</td>
<td>Poor compatibility with mass spectrometry due to high salt concentration</td>
<td>3</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>High resolution</td>
<td>Samples typically need derivitation</td>
<td>4</td>
</tr>
</tbody>
</table>

My goal was to develop a LC-MS/MS method that could:

- Differentiate diverse characteristics.
- Be label-free.
- Require minimum sample pretreatment.
- Be compatible with mass spectrometry.
- Establish an in-house ms/ms library.
- Be capable of analyzing industrially relevant samples containing a mixture of sugars.
- Be high throughput.
An LC-MS/MS method was developed; over 70 sugars derived from lignocellulose with different sizes, polarity, acidity and linkage positions were identified.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mode</th>
<th>Precursors</th>
<th>NCE</th>
<th>Arb</th>
<th>[M+COOH]⁻</th>
<th>[M-H]⁻</th>
<th>[2M-H]⁻</th>
<th>[M + Cl]⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose n=2</td>
<td></td>
<td>100.00%</td>
<td>9.50%±0.3%</td>
<td>0.49%±0.1%</td>
<td>0.04%±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose n=6</td>
<td></td>
<td>100.00%</td>
<td>0.6%</td>
<td>0.30%±0.1%</td>
<td>4.00%±0.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylobiose n=6</td>
<td></td>
<td>100.00%</td>
<td>5.79%±0.1%</td>
<td>2.48%±0.2%</td>
<td>3.46%±0.2%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylotriose n=6</td>
<td></td>
<td>100.00%</td>
<td>1.91%±0.1%</td>
<td>2.83%±0.6%</td>
<td>5.73%±1.0%</td>
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<td></td>
<td></td>
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<tr>
<td>Xylotetraose n=6</td>
<td></td>
<td>100.00%</td>
<td>3.60%±0.3%</td>
<td>NC</td>
<td>7.50%±0.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylopentaose n=6</td>
<td></td>
<td>100.00%</td>
<td>4.86%±0.3%</td>
<td>NC</td>
<td>8.03%±0.8%</td>
<td></td>
<td></td>
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<tr>
<td>Xylohexaose n=6</td>
<td></td>
<td>100.00%</td>
<td>9.70%±0.4%</td>
<td>NC</td>
<td>8.56%±1.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-X2 n=1</td>
<td></td>
<td>100.00%</td>
<td>6.1%</td>
<td>6.11%</td>
<td>4.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-X3 n=1</td>
<td></td>
<td>100.00%</td>
<td>2.0%</td>
<td>NC</td>
<td>11.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucuronic Acid n=2</td>
<td></td>
<td>0.25%±</td>
<td>42.50%±</td>
<td>0.54%±0.1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U⁻²m²XX n=4</td>
<td></td>
<td>0.06%</td>
<td>100.00%</td>
<td>40.31%</td>
<td>0.15%±0.1%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XU⁻²m²XX n=2</td>
<td></td>
<td>ND</td>
<td>100.00%</td>
<td>NC</td>
<td>0.10%±</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

MS/MS spectra of U⁻²m²XX targeting precursor [M-H]- with varying collision energy
Application of the established LC-MS/MS method to characterize industrial sample before and after enzyme treatment

<table>
<thead>
<tr>
<th>Compound</th>
<th>Untreated</th>
<th>Enzymes cocktail containing AxyAgu115A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Xylobiose</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Xylotriose</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Xylotetraose</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Xyloctaose</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Xylohexaose</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>MeGlcA</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>U^{4m2}X</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>U^{4m2}XX</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>XU^{4m2}XX</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>
Engineering significance

• SdeAgu115A and AxyAgu115A have the potential to tailor xylans with high molecular weight.
• The unique tolerance properties of these two enzymes can benefit industrial applications with corresponding conditions.
• AxyAgu115A was active on xylan recovered from liquid hot water extraction of mixed hardwood.
• An in-house LC-MS/MS method was developed to characterize the hemicellulosic fraction before and after enzyme treatment, which has the potential to be applied to other plant fibers.
Acknowledgements

**Fiber characterization**

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**University of Guadalajara, Mexico**  
Prof. G. Toriz

**University of Helsinki, Finland**  
Prof. M. Tenkanen

**Synergy**

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**Chalmers University of Technology, Sweden**  
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**University of Guadalajara, Mexico**  
Prof G. Toriz

**University of Helsinki, Finland**  
Prof. M. Tenkanen

**Application**

**University of Toronto, Canada**  
A. Gaona, Prof. B. Saville, T. Oakes, F. Razeq, Prof. E. Master, Dr. A. Starostine

**University of Helsinki, Finland**  
Prof. M. Tenkanen

**Publication**

Direct and up-close views of plant cell walls show a leading role for lignin-modifying enzymes on ensuing xylanases

**Publication**

Biochemical and structural characterization of a five-domain GH115 alpha-glucuronidase from the marine bacterium *Saccharophagus degradans* 2-40T

Action of a GH115 α-glucuronidase from *Amphibacillus xylinus* at alkaline condition promotes release of 4-O-methylglucopyranosyluronic acid subunits of glucuronoxylan and arabinoglucuronoxylan

**Manuscript to be submitted**

Synergistic action of accessory hemicellulases from glycoside hydrolase families GH115, GH62, and GH51 towards spruce arabinoglucuronoxylan

**Ongoing Project**

LC-MS/MS method development and application of GH115 α-glucuronidase AxyAgu115A, to process glucuronoxylan released through hot-water extraction of mixed hardwood prior to steam-explosion

