Enzyme tools and carbohydrate tools for bioproducts development

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Committee members: Professor Bradley Saville and Professor Alexander Yakunin

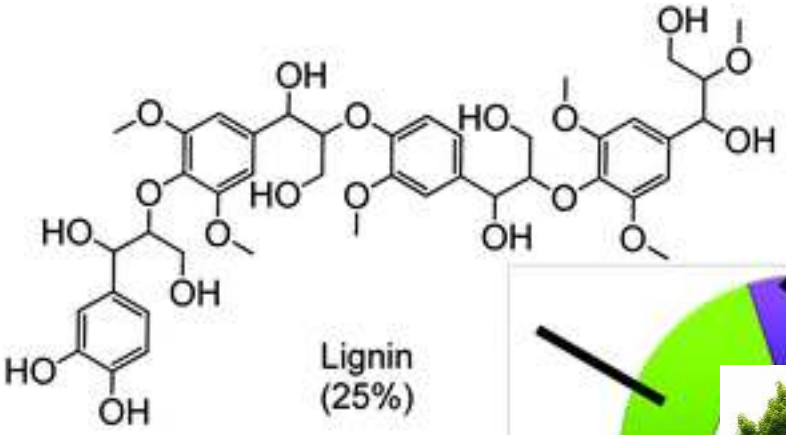
Levulinic acids

Bioethanol

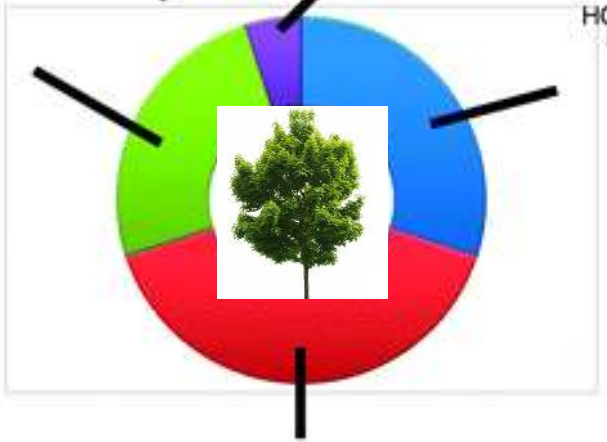
Heat and power

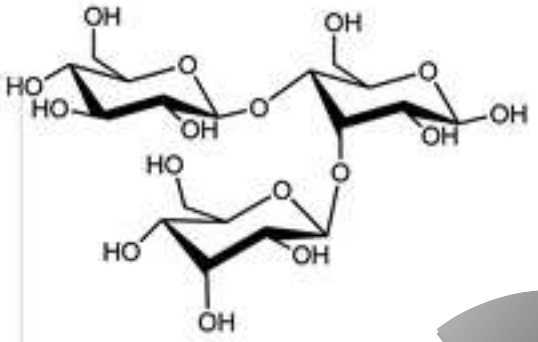
Formic acids

Deconstructi
on or
combustion



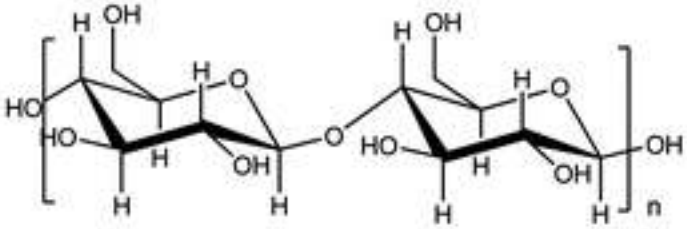
Lignin
(25%)





Hemicellulose
(30%)

Enzymatic
modificatio

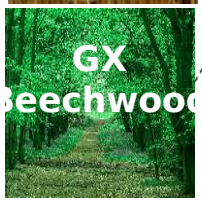


Cellulose
(40%)

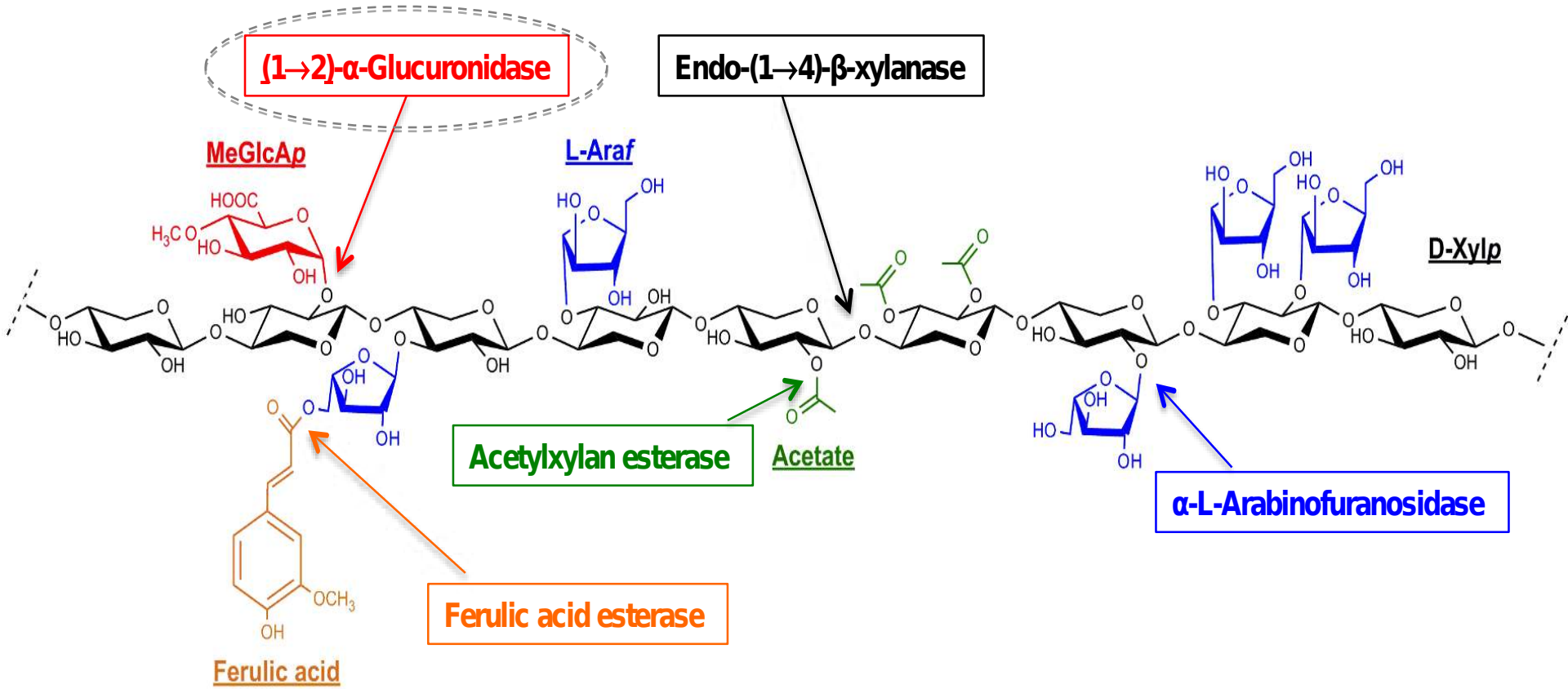
Biomaterials

Chem. Soc. Rev., 2014, 43, 7485-7500

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, carbonhydrate-active enzymes are produced in organisms from various habitats including harsh environmental conditions

http://australianfungi.blogspot.ca

http://wallpapersin4k.net

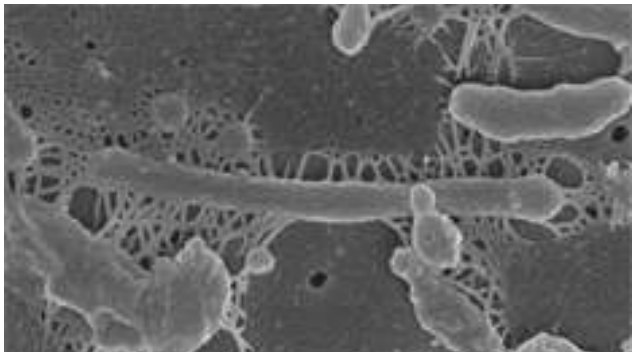
https://en.wikipedia.org/wiki/Iceberg

http://www.bugoutservice.com/termite-protection

Select bacteria from unique habitats

Salt marsh cord grass in the Chesapeake Bay

Marine bacterium *Saccharophagus degradans*

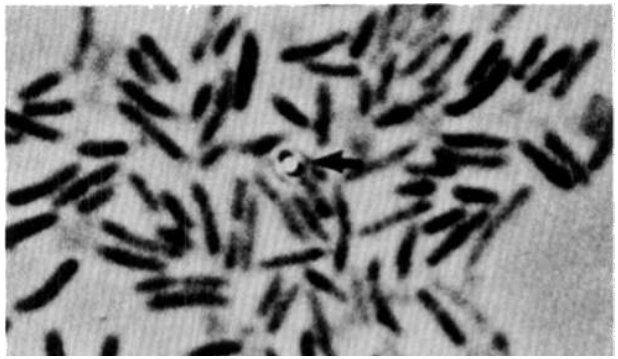


https://microbewiki.kenyon.edu

Tolerance in high salt condition?

Composts of manure with grass and rice straw in Japan

Alkaliphilic bacterium *Amphibacillus xylanus*

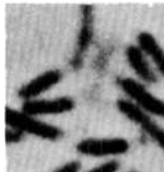


https://microbewiki.kenyon.edu

Tolerance in alkaline condition?

Produce enzymes using biotechnology

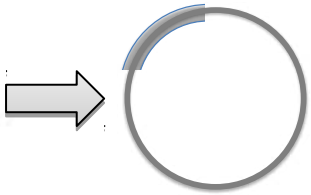
ource organism



ATCGCTAGTACG
GCATGCACTGTG
CATAATTCCAGTA
CGTTTGGGATCG

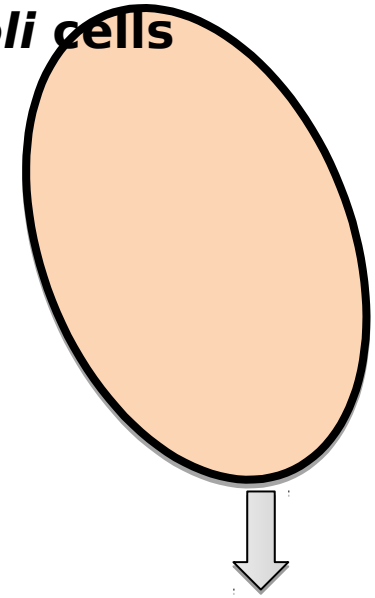
Obtain gene of interest through genomic DNA or direct synthesis

Amplificati on

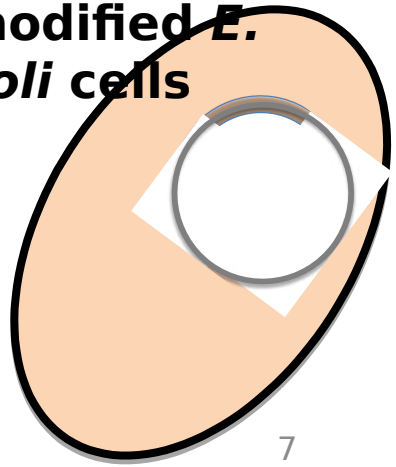


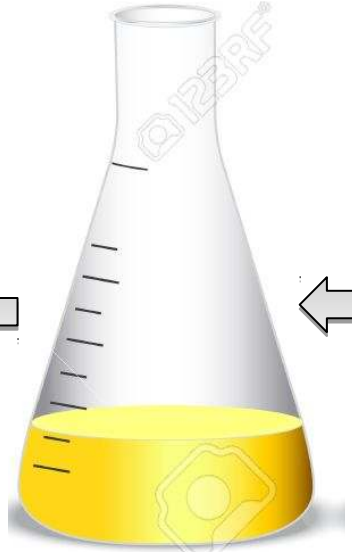
Insert into plasmid

Transform *E. coli* cells



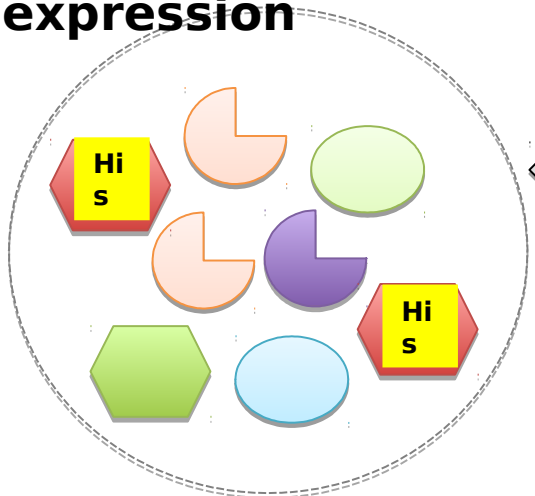
Genetically modified *E. coli* cells

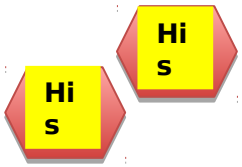




Bacterial culture

Induce protein expression



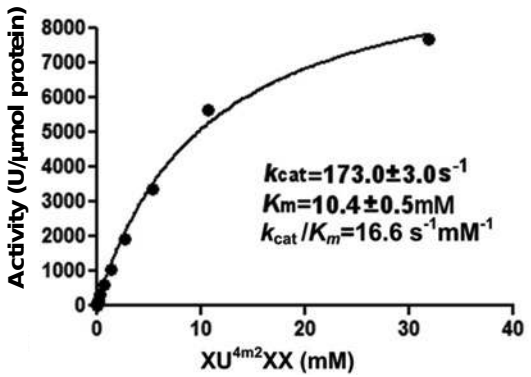


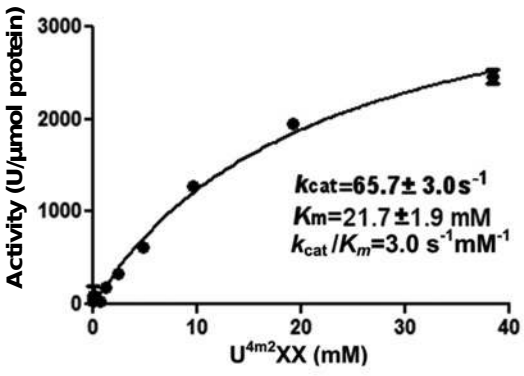
Enzyme purification

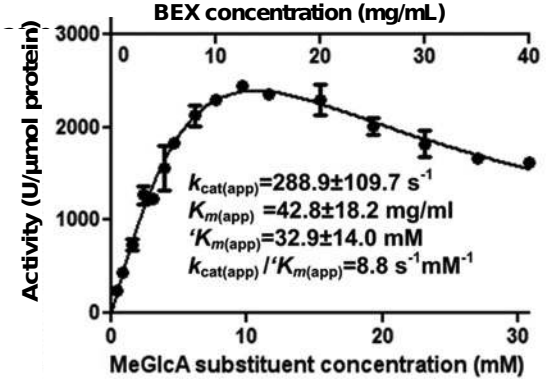
Both enzymes demonstrated activity towards glucuronoxylans and oligomers with preference towards internally substituted residues

BEX, beechwood glucuronoxylan

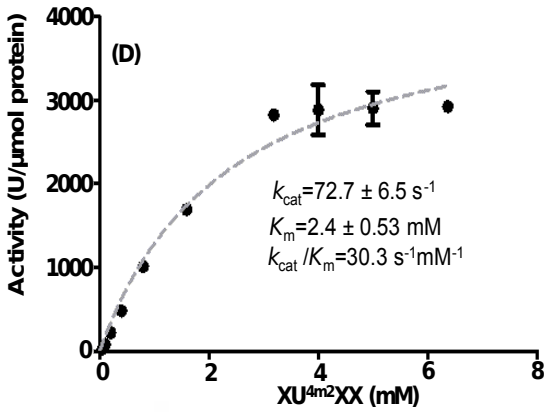
SdeAgu115A

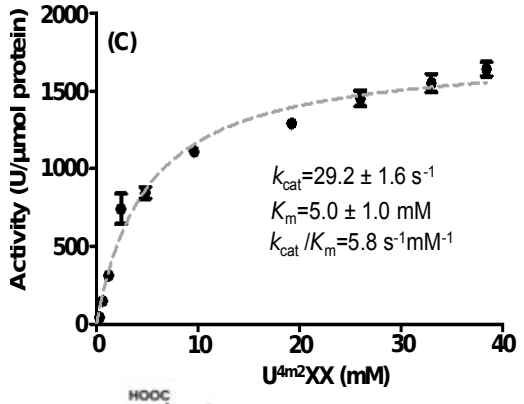


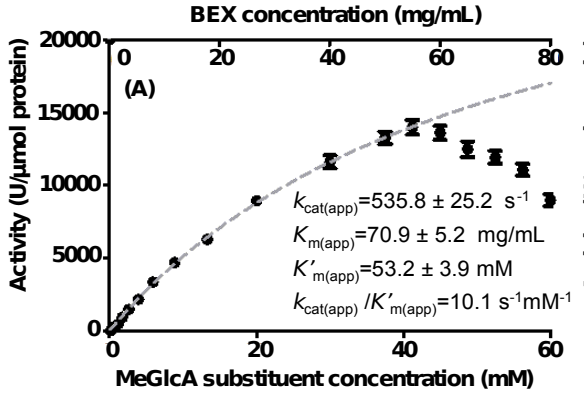


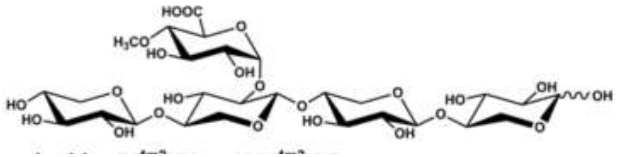


AxyAgu115A

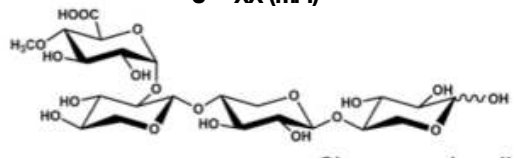








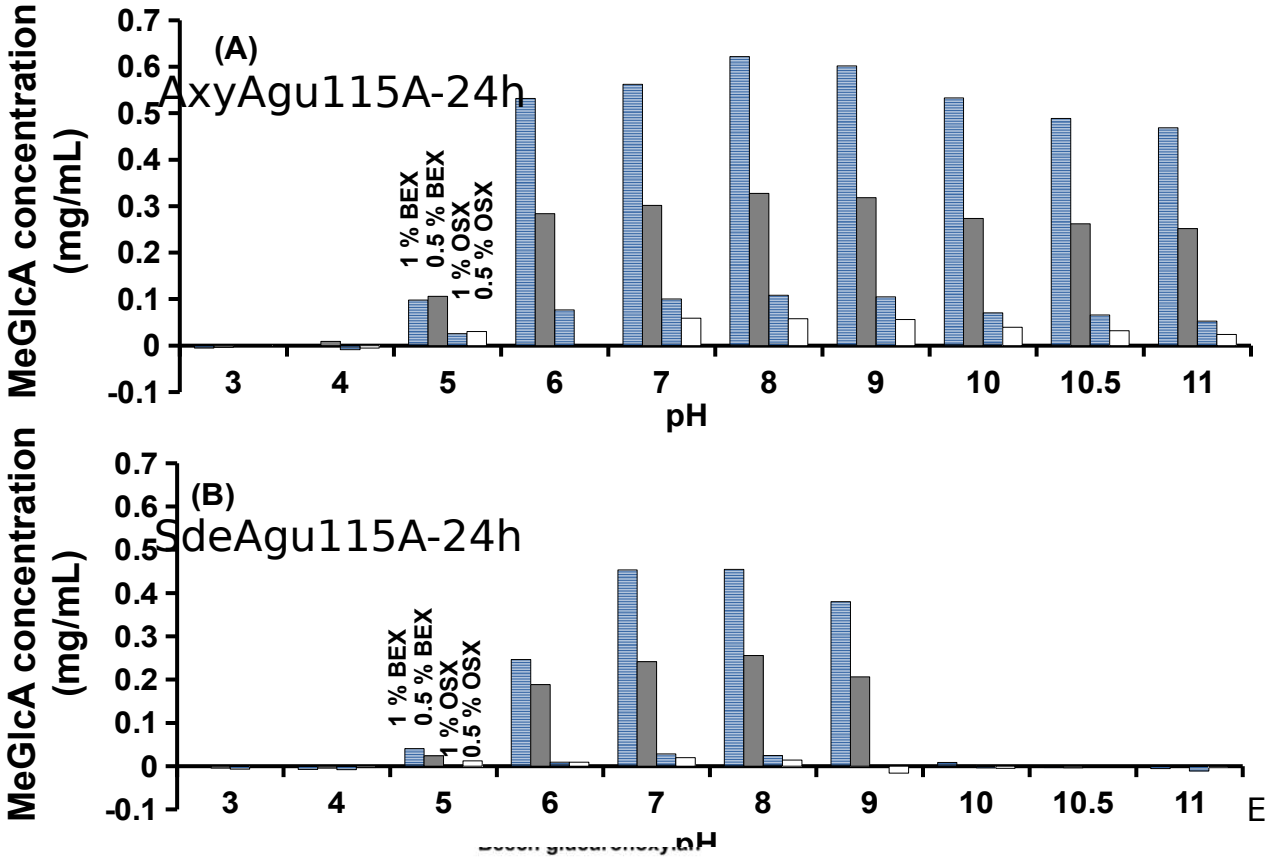
XU4m2XX, aldopentauronic acid



U4m2XX, aldotetrauronic acid

J. Biol. Chem. 291 (2016) 14120-
Enzyme Microb. Technol. 104 (2017) 22-

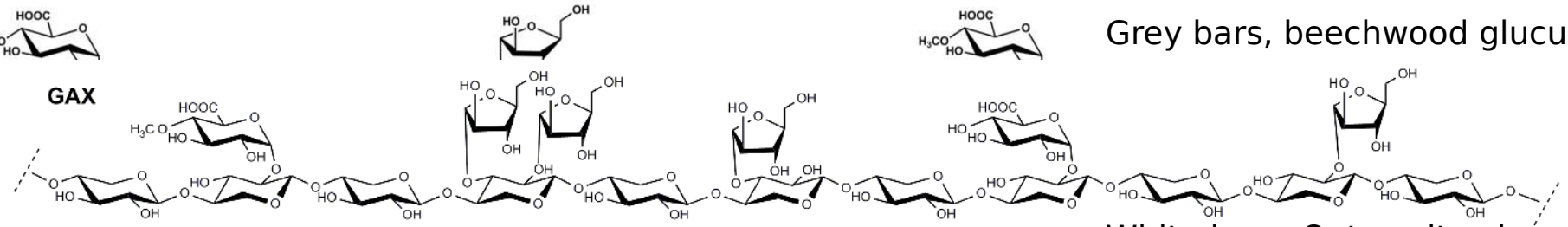
AxyAgu115A demonstrated better performance in alkaline condition



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

Increase in substrate solubility in alkaline condition increased substrate accessibility.

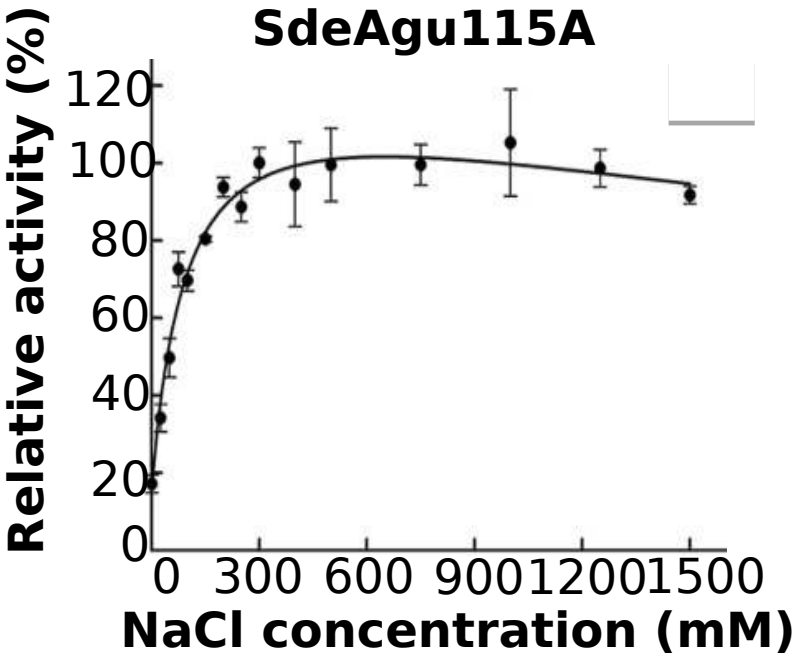
Enzyme Microb. Technol. 104 (2017) 22–2

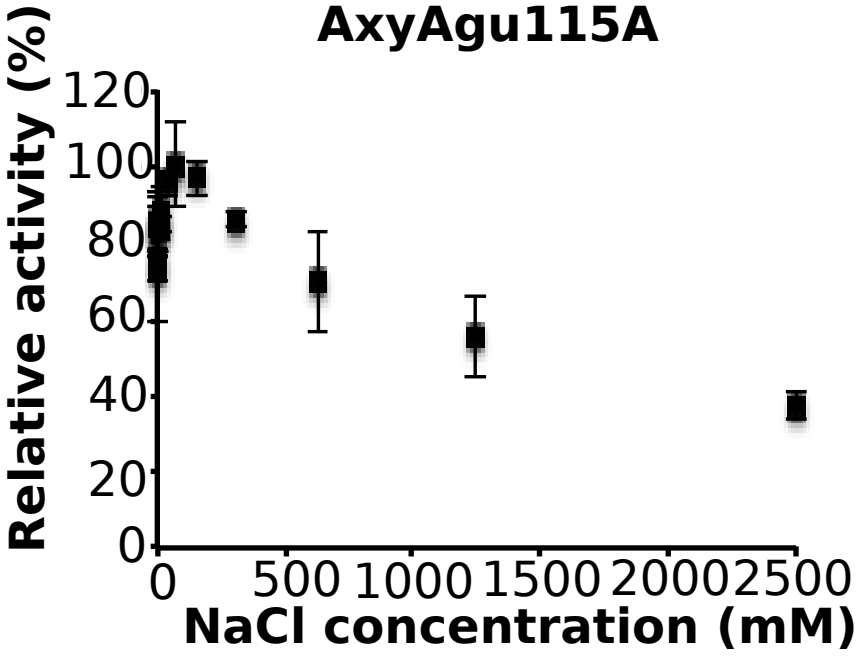


Grey bars, beechwood glucuronoxy

White bars, Oat spelt xylan

Consistent with the marine origin of SdeAgu115A, salt activation of enzyme activity was observed for SdeAgu115A but not AxyAgu115A



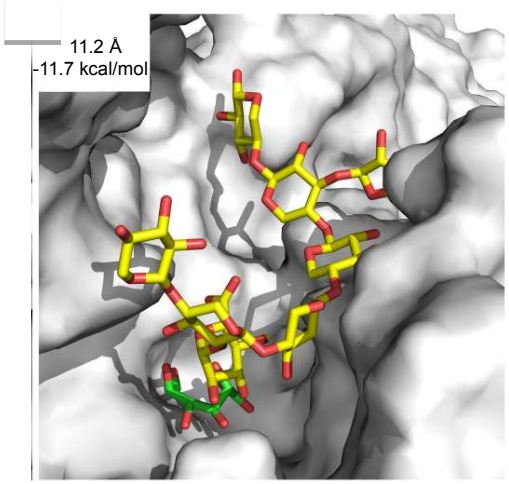


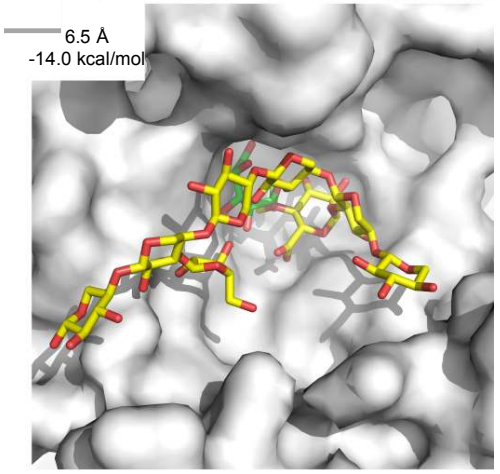
J. Biol. Chem. 291 (2016) 14120-
Enzyme Microb. Technol. 104 (2017) 22-

AxyAgu115A displayed higher activity towards complex xylans compared to SdeAgu115A

Polymeric substrates	Activity (µmol product/min/µmol enzyme)	
	SdeAgu115A	AxyAgu115A
Beechwood glucuronoxylan	2470 ± 70	4700 ± 100
Spruce arabinoglucuronoxylan	917 ± 6	5630 ± 60
Oat spelt glucuronoarabinoxylan	24 ± 1	501 ± 12

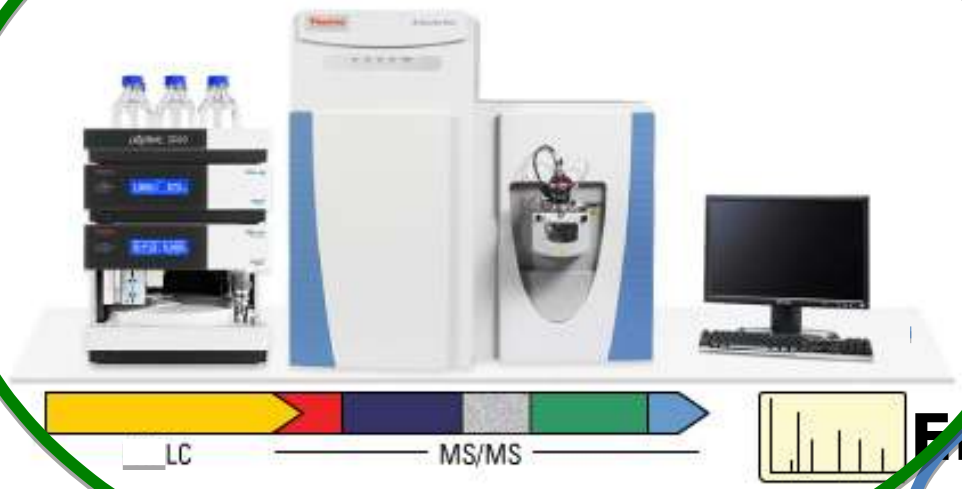
- Accommodation of complex xylans was consistent with docking analysis that predicted accessibility of AxyAgu115A to branched xylo-oligosaccharides.





Enzyme Microb. Technol. 104 (2017) 22–28.

LC-MS/MS



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

Colorimetric a sis

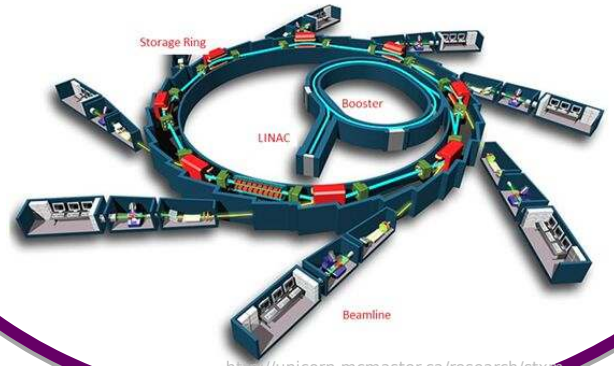
Enzyme coupling metho

$$\text{Glucuronoxylan} + H_2O \xrightarrow{\text{GH115}} \text{Xylan} + \text{GlcA}$$

$$\text{GlcA} + NAD^+ + H_2O \xrightarrow{\text{Uronate dehydrogenase}} \text{Glucarate} + NADH + H^+$$

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

Scanning transmission X-



https://unicorn.mcmaster.ca/research/stxm-intro/polySTXMintro-all.html

Thin layer chromatogra

https://www.spectrumchemical.com

Pros and cons of existing techniques

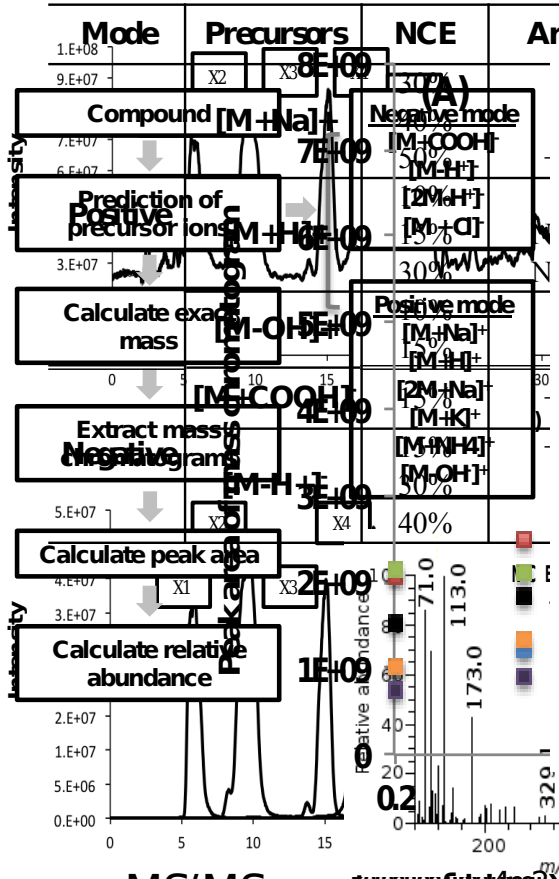
Separation techniques	Pros	Cons	Ref
Size exclusion	Identified analytes above 9 kDa	Compounds with the same size co-elute	1
Ion chromatography	Separated neutral and acidic oligomers	Substitution position of MeGlcA was not confirmed	2
HPAEC-PAD	Effective separation of oligomers	Poor compatibility with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

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.

Total ion/mass chromatogram → Relative abundance distribution → In-house library → Quantitative assessment



Sample	X1 X2 X3 X4 X5 X6 GlcA [M+COOH]⁻	[M-H⁺]⁻	[2M-H⁺]⁻	U^{4m2}XX XU^{4m2}XX [M + Cl]⁻
Arabinose $^{n=2}$	100.00%	9.50% ± 0.3%	0.49% ± 0.1%	0.04% ± 0.03%
Xylose $^{n=6}$	100.00%	11.65% ± 0.6%	0.30% ± 0.1%	4.00% ± 0.5%
Xylobiose $^{n=6}$	100.00%	5.79% ± 0.1%	2.48% ± 0.2%	3.46% ± 0.2%
Xylotriose $^{n=6}$	100.00%	1.91% ± 0.1%	2.83% ± 0.6%	5.73% ± 1.0%
Xylotetraose $^{n=6}$	100.00%	3.60% ± 0.3%	NC	7.50% ± 0.8%
Xylopentaose $^{n=6}$	100.00%	4.86% ± 0.3%	NC	8.03% ± 0.8%
Xylohexaose $^{n=6}$	100.00%	9.70% ± 0.4%	NC	8.56% ± 1.3%
A-X2 $^{n=1}$	100.00%	6.1%	6.11%	4.3%
A-X3 $^{n=1}$	100.00%	2.0%	NC	11.0%
Glucuronic Acid $^{n=2}$	0.25% ± 0.06%	100.00%	42.50% ± 40.31%	0.54% ± 0.1%
U^{4m2}XX $^{n=4}$	ND	100.00%	NC	0.15% ± 0.1%
XU^{4m2}XX $^{n=2}$	ND	100.00%	NC	0.10% ± 0.02%
U XXX$_m$	37.3 ± 0.1	735.2201	[M-H⁺]	

MS/MS spectra of U^{4m2}XX 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

Compound	**Untreated**	**Enzymes cocktail containing AxyAgu115A**
Xylose	++	+++
Xylobiose	++	++
Xylotriose	+++	++++
Xylotetraose	+++	++++
Xylopentaose	+++	++++
Xylohexaose	+++	++++
MeGlcA	+	++++
$U^{4m2}X$	+++	++
$U^{4m2}XX$	+++	++
$XU^{4m2}XX$	+++	++

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

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Fiber characterization

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Prof. E. Master
Mississippi State University, United States
Prof. D. Jeremic
Niagara University, United States
Prof. R. Goacher
Canadian Light Source Inc, Canada
C. Karunakaran (Science manager)

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

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Enzyme characterization

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The Structure Biology Center, United States
Dr. B. Nocek
Chalmers University of Technology, Sweden
Prof. P. Gatenholm
University of Guadalajara, Mexico
Prof. G, Toriz
University of Helsinki, Finland
Prof. M. Tenkanen

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 xylanus* at alkaline condition promotes release of 4-O-methylglucopyranosyluronic acid subunits of glucuronoxylan and arabinoglucuronoxylan

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University of Helsinki, Finland
Prof. M. Tenkanen

→ ***Manuscript to be submitted***
Synergistic action of accessory hemicellulases from glycoside hydrolase families GH115, GH62, and GH51 towards spruce arabinoglucuronoxylan

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Dr. W. Wang
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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

→ ***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

Dr. A. Starostine,
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Ms. C. Hong
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