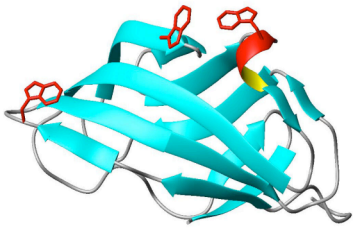
Enzymatic Valorization of Plant Fibres and Polymers

Emma R. Master
Chemical Engineering & Applied Chemistry
University of Toronto



http://chem-eng.utoronto.ca/~bioproducts

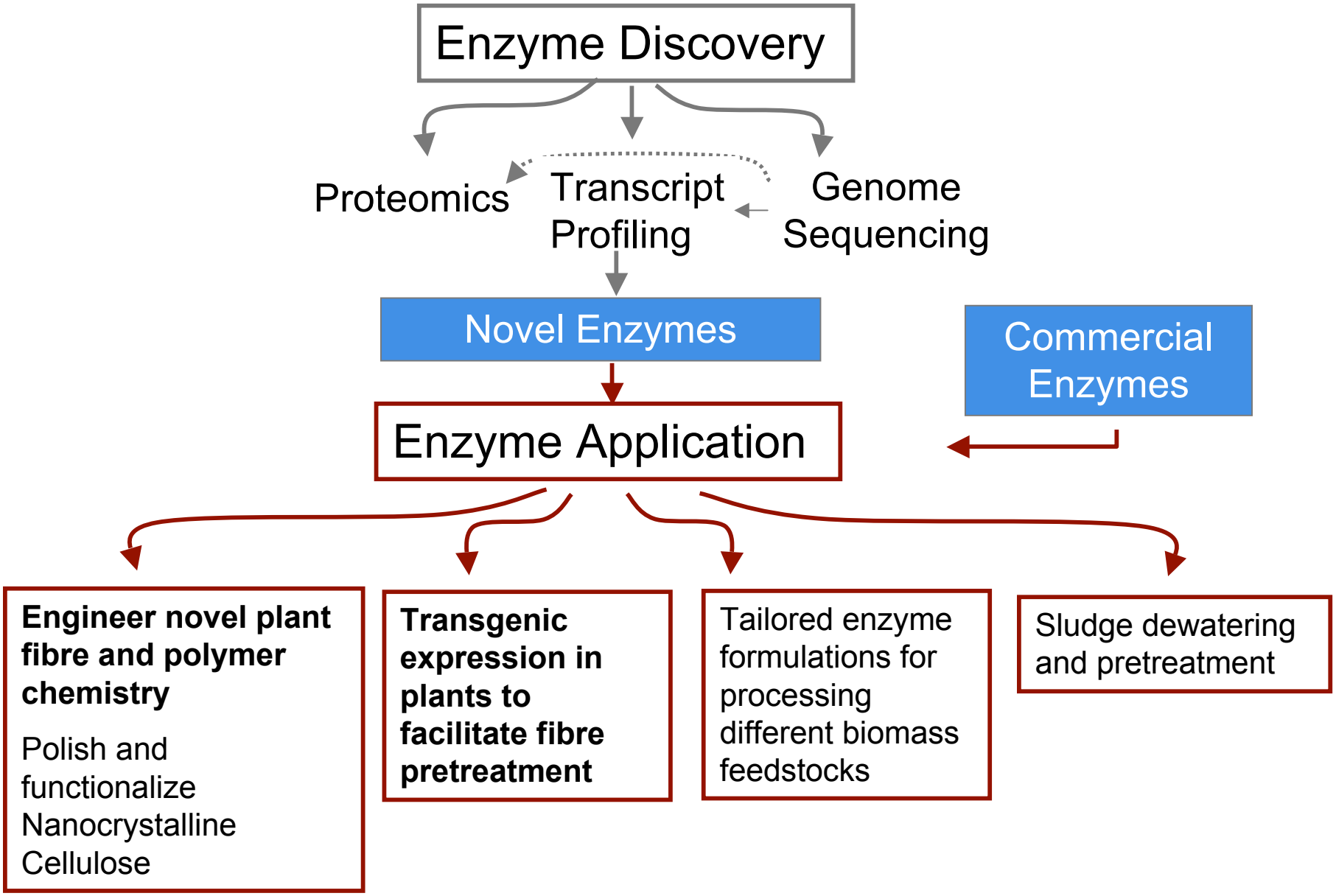
Why focus on high-value co-products and enzyme catalysts?

- Off-set biofuel costs
- New markets for forest and agriculture sectors

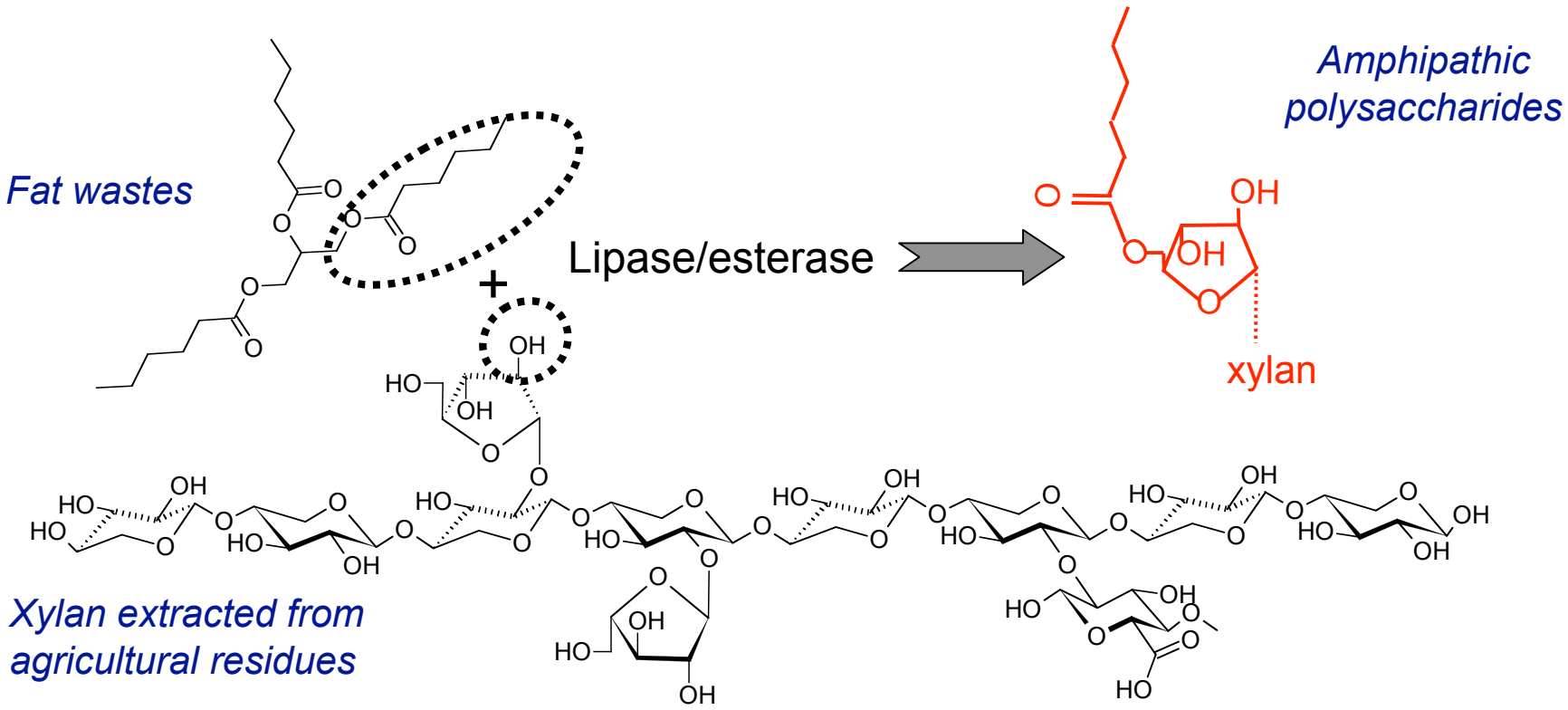
Valued enzyme properties

- Mild reaction reactions
- Regio- and stereo- specificity = production of chiral compounds from heterogenous substrates

Our Research Activities



1. Enzyme-catalyzed Transesterification of Polysaccharides and Lignin-derived Phenolic Acids

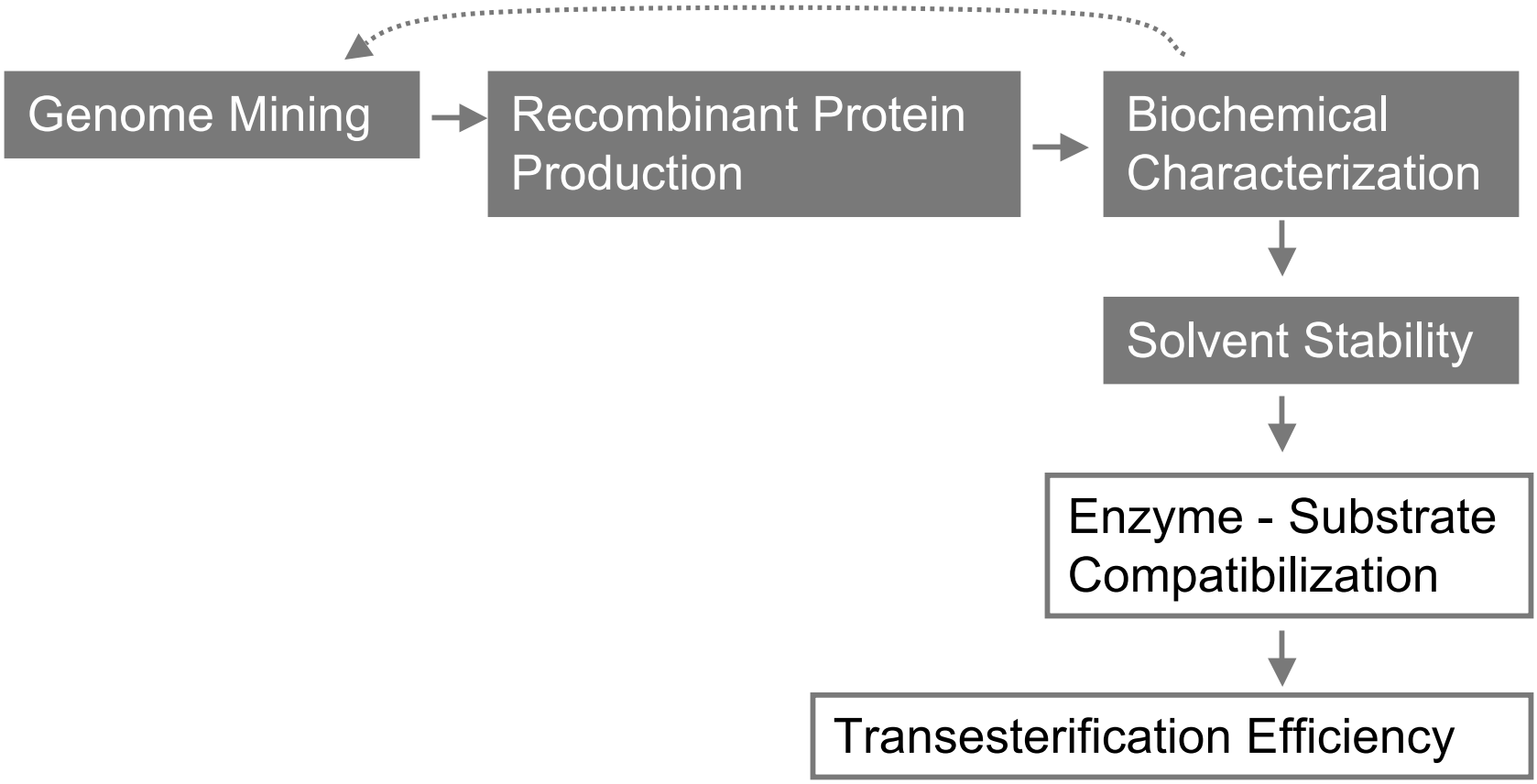


Example Applications:

- Edible films for food packaging
- Water-repellent cellulose coatings
- Biodegradable surfactants
- Lipophilic phenolic acids

Enzyme-catalyzed Transesterification: Process Requirements

- Solvent-stable esterases and/or lipases are required
- Our specific aim: isolate highly stable arylesterases



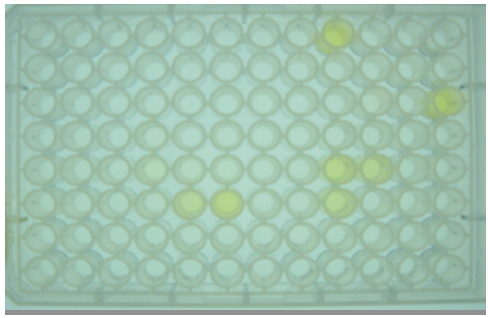
Enzyme Discovery Through Bacterial and Archaeal Genome Mining

Activity	Targeted	Cloned to date	Expressed and Purified to date	Characterized to date
Esterase/lipase	219	190	97	88

HTP Primary screens

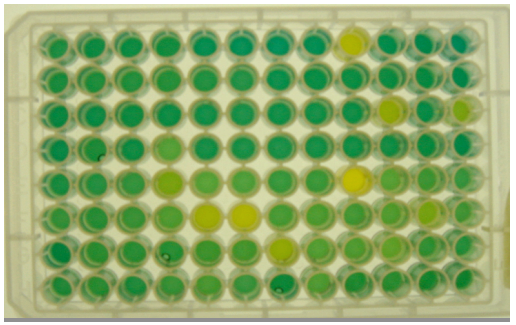
(49 of 88 purified proteins showed activity)

pNP acetate (C2)
pNP butyrate (C4)
pNP caproate (C10)
pNP laurate (C12)
pNP palmitate (C18)
pNP benzoate

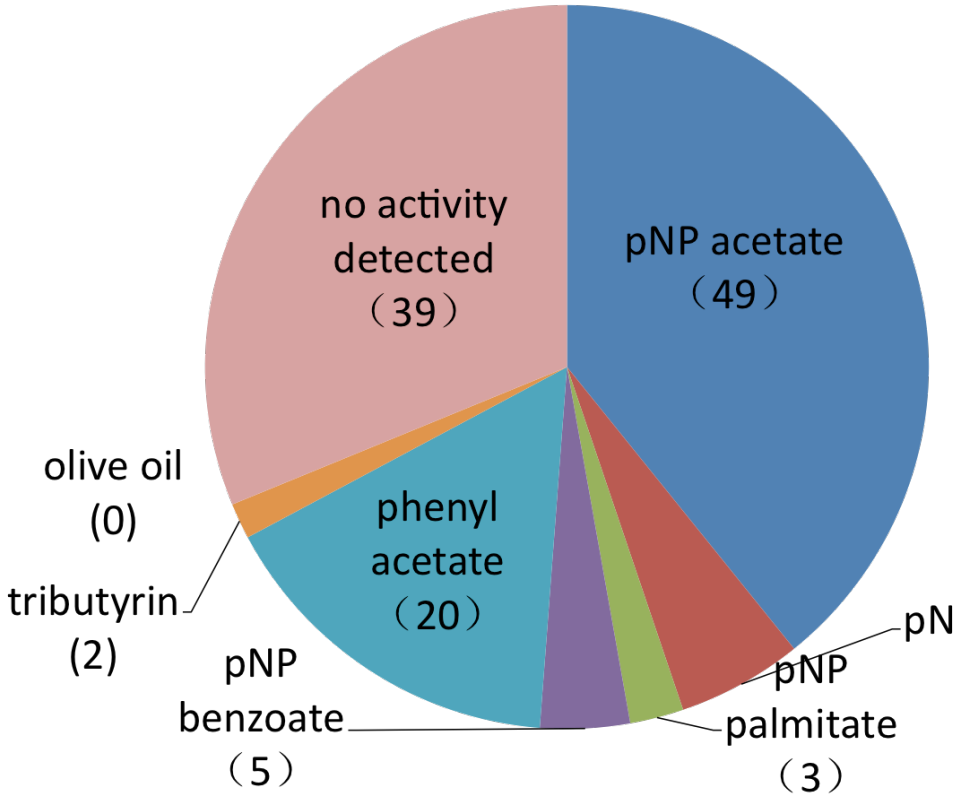


Phenyl acetate
Tributyrin
Olive oil

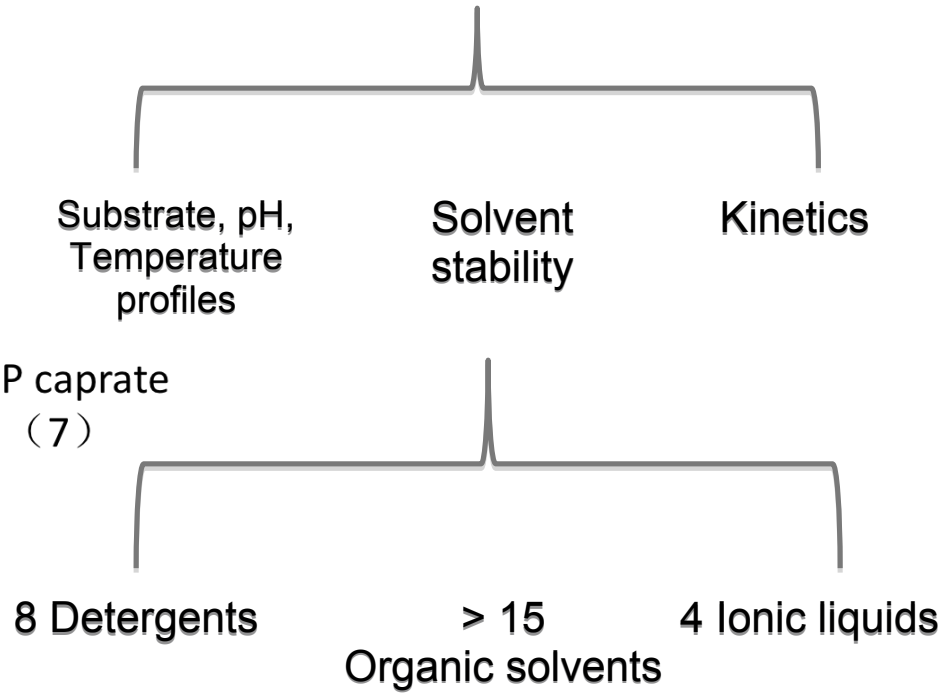
Agar plates + Rhodamine B or BTB assay



Secondary Screens for Arylesterases



3 esterases from *Rhodopseudomonas palustris*
3 esterases from *Pseudomonas putida*
1 esterase from *Streptomyces avermitilis*
1 esterase from *Pseudomonas aerigonosa*
2 esterases from *Archaeoglobus fulgidus*

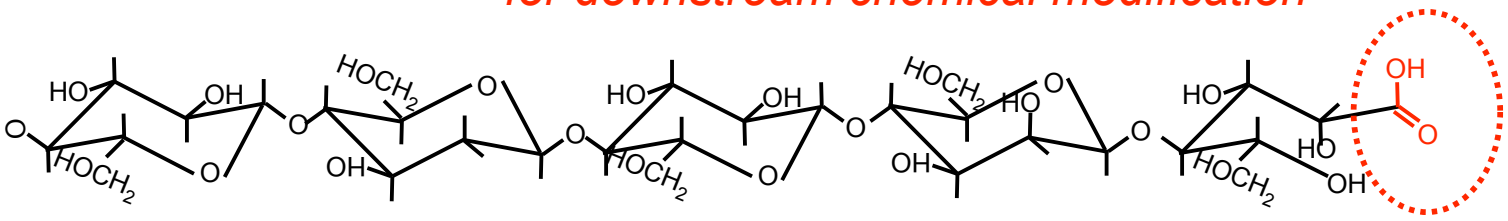


Representative Data: Two Novel Arylesterases from *A. fulgidus*

Properties	AF_Est1	AF_Est2
Molecular Weight	30.2kDa	30.3kDa
Specific Activity on pNP acetate (at 37°C)	0.74	0.81
Specific Activity on phenyl acetate (at 37°C)	7.0	7.9
Optimal pH	pH 9.5	pH 9-10
pH stability (residual activity over 80%)	pH 7-11	pH 7-11
Half life at 80°C	> 5 h	> 5 h
Stability in 50 % of organic solvents	All except isopropanol, tetrahydrofuran	All except acetonitrile, isopropanol, tetrahydrofuran
Stability in > 40% of Ionic liquids	70%-100% $[BMIM]BF_4$, 60%-70% $[BMIM]PF_6$, 60%-100% $[BMIM]CF_3SO_3$	70%-100% $[BMIM]BF_4$, 60%-100% $[BMIM]CF_3SO_3$

2. Chemo-enzymatic Modification of Cellulose

Enzymatic "Activation" of polysaccharides for downstream chemical modification

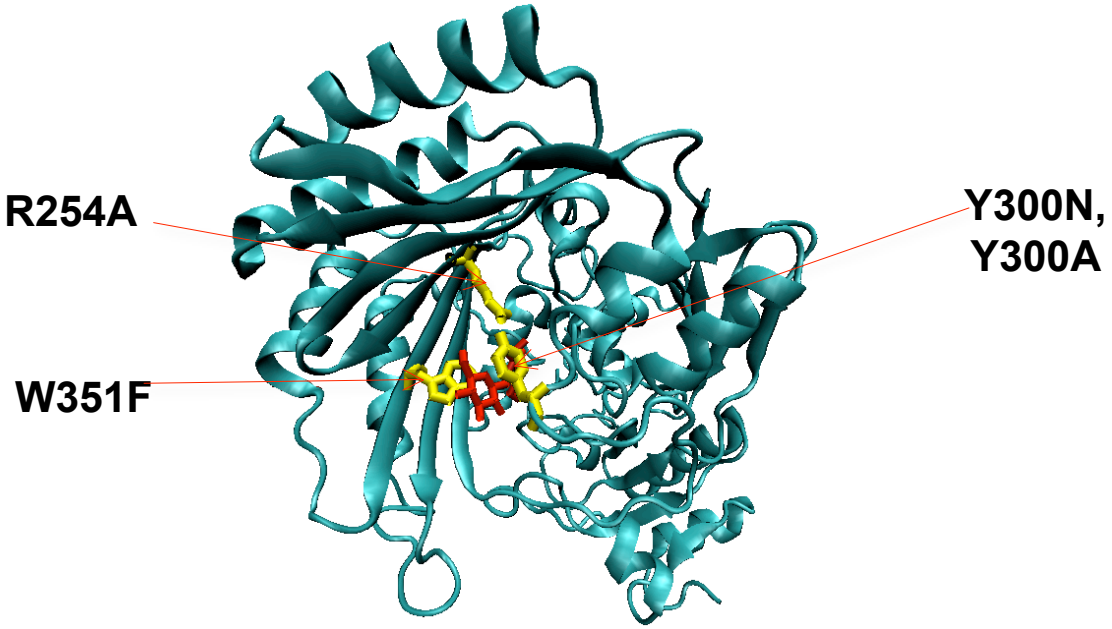


Benefits of Enzyme catalysts:

• High specificity and low energy requirements allow discrete and reproducible modification of pulp fibre with minimal impact on fibre length or viscosity.

• Production of chiral compounds is facilitated.

Enzyme Target: Glucooligosaccharide oxidase



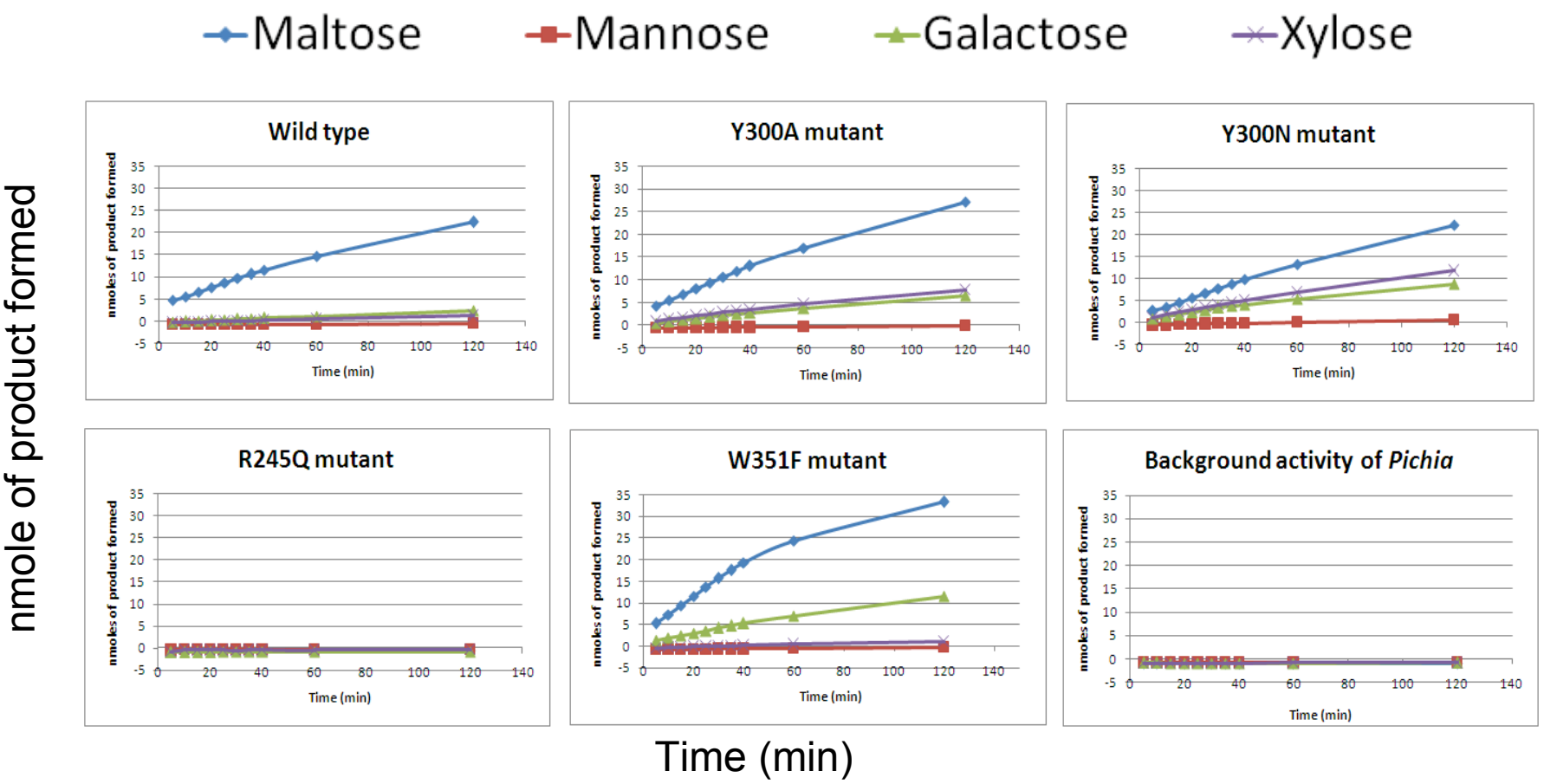
Objectives:

•Site directed mutagenesis of GOOX to expand substrate specificity

• Production of chimeric proteins containing GOOX and CBMs to increase GOOX activity on polymeric substrates

Site-directed mutagenesis and production of chimeric enzymes → Recombinant Protein Production → Biochemical Characterization

Increased Substrate Specificity of GOOX



Current test substrates: cellobiose, cellotriose, cellotetraose, xylobiose, xylotriose, maltobiose, maltotriose, chitobiose, chitotriose

Measure impact of CBM3, CBM11, and CBM44

3. *In vivo* Fibre Engineering

Our aim is to:

- Increase enzyme accessibility to fibre components
- “Prime” biomass feedstock for pretreatment

Approach:

Target hemicellulases that hydrolyze intermolecular linkages between xylan and lignin in an effort to “loosen” the lignocellulose network before a plant is harvested and processed.

Bacterial Glycoside Hydrolase Targets

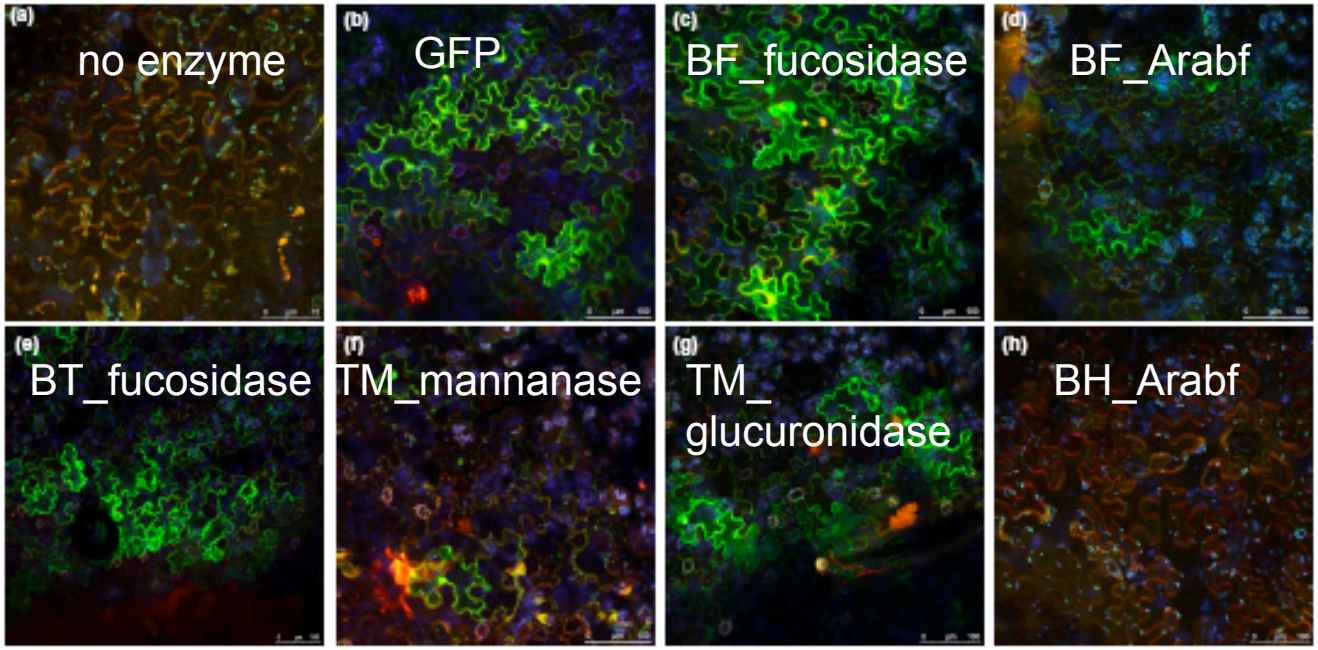
Activity	Targeted	Cloned to date	Soluble Expression and Purification	Characterized to date	Selected for Transgenic Expression
Mannanase (GH5)	26	18	8	3	2
α-glucuronidase (GH67)	2	1	1	1	1
α-arabinofuranosidase (GH51)	46	31	11	3	7
α-fucosidase (GH29)	24	17	2	2	2
α-galactosidase	5	2	-	-	3

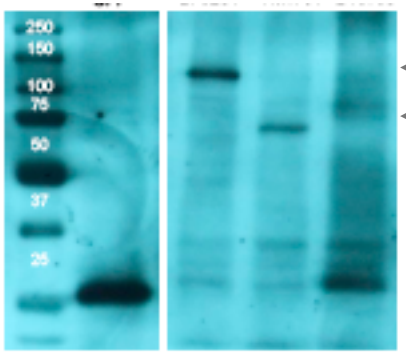
Also… fungal targets: feruloyl esterase, acetyl xylan esterase, glucuronoyl esterase

Transient Expression Pipeline

35S Promoter	Glycosyl hydrolases	Green Fluorescence Protein

Infiltration using Agrobacterium





BF_fucosidase (98 KDa)

TM_mannanase (64 KDa)

GFP (27 KDa)

BF_Arabf/ GLP only?

Transgenic Expression in Arabidopsis

GH Construct:

Activator line (target expression to late vascular tissue):

Fibre analyses:

- Total sugar
- FT-IR and Raman
- Fibre thickness, length, shape, and fibril angle (EvaluTree - Hi Resolution Fibre Quality Analyzer)
- Pretreatability

Summary

• High value co-products from biomass is a important means of reducing biofuel costs in the near-term, while ensuring the sustainability of biorefineries in the long-term.

• Enzymes are ideal catalysts for plant fibre and polymer engineering given their specificity and performance in comparatively mild reaction conditions.

Three examples:

- Transesterification of oligosaccaharides and lignin-derived aromatic compounds
- Oxidative activation of cellulose and hemicellulose
- Transgenic expression of accessory enzymes

Acknowledgements

•Lijun Wang
•Maryam Foumani
•Alex Tsai
•Tom Canam
•Prof. M. Campbell (CSB, UofT)

• Alberta Research Council
• FPInnovations
• Structural Proteomics in Toronto (SPiT)

• NSERC
• Ontario Ministry of Research and Innovation