

Genomic & proteomic approaches to identify & characterize fungal biomass-degrading enzymes

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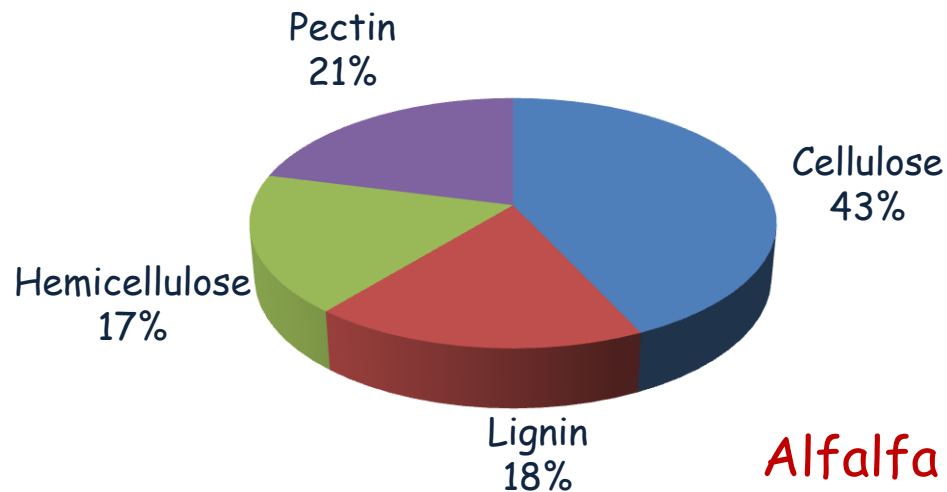
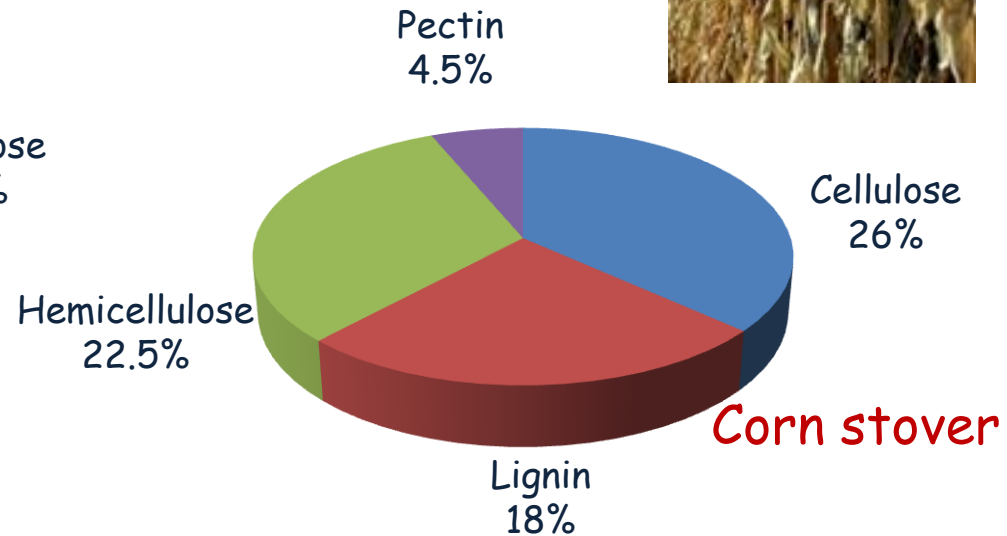
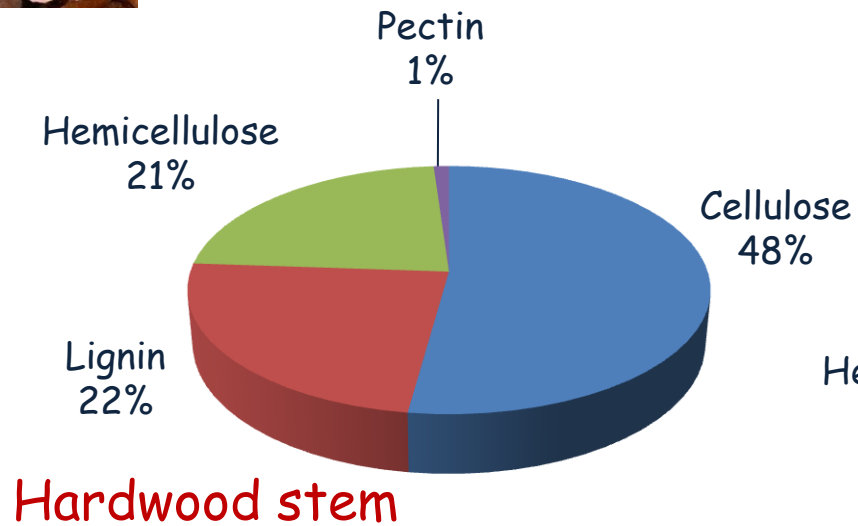


Topics addressed

- Basics of enzymatic degradation of lignocellulose
- The need for efficient enzymes and enzyme cocktails
- Diversity of fungal degrading enzymes
- Genomic and proteomic approaches to characterize enzymes and develop enzyme cocktails

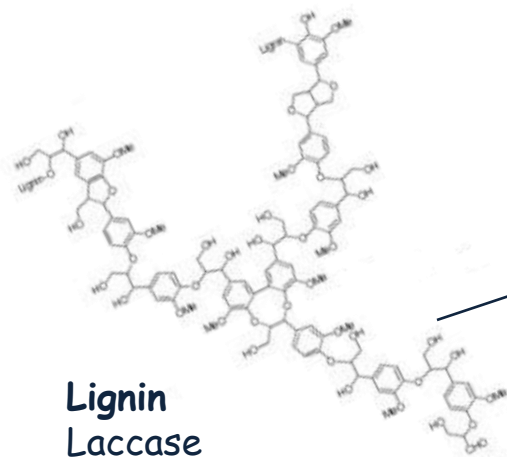


Feedstock composition varies

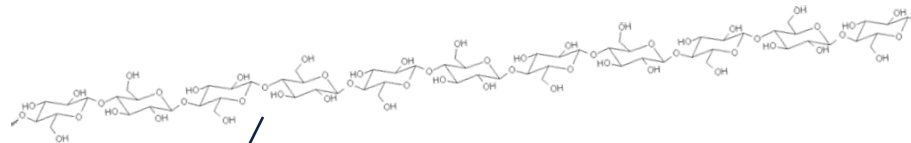




Lignocellulose-active proteins

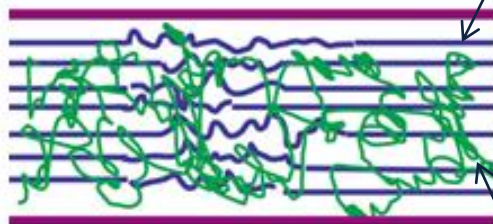


Lignin
Laccase
lignin peroxidase
manganese peroxidase

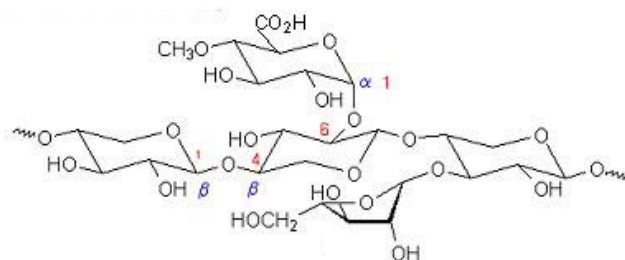


Cellulose

- Endoglucanase, exoglucanase, beta-glucosidase
- Sowellennin, expansin

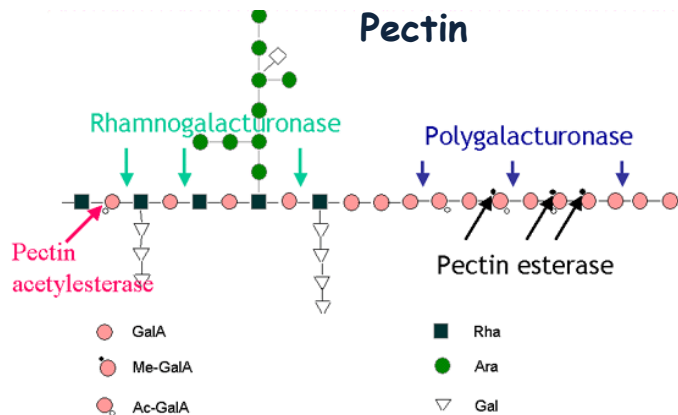


Lignocellulose



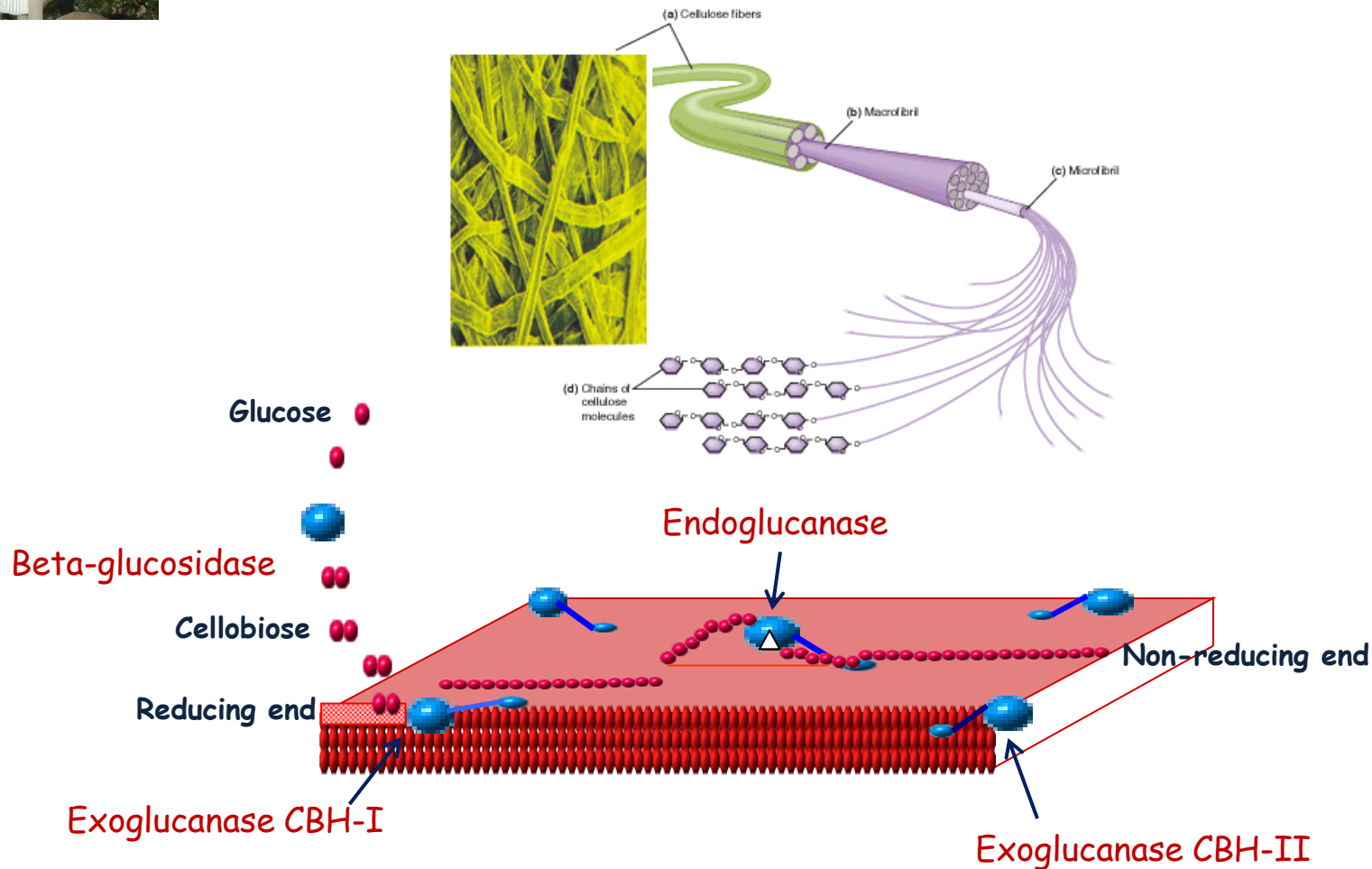
Hemicellulose

Xylan - endoxylanase, xylosidase, acetyl-xylanesterase, feruloyl esterase, alpha-glucuronidase,
Mannan - mannanase, mannosidase
Arabinan - arabinofuranosidase, arabinase,





Cellulase refers to a mixture of 4 distinct enzyme activities



Other, unknown proteins are required for efficient digestion of crystalline cellulose



Enzyme cost



Corn starch

Amylase / glucoamylase
0.26 g of protein (\$0.03)



Straw

Cellulase, 26 g of protein
(\$0.25*)



glucose



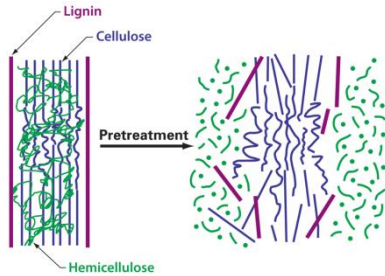
Source: US DOE National Renewable Energy Laboratory ; *economic viability, \$0.04



Commercial cellulase

- Cellucast (*Trichoderma reesei*) and beta-glucosidase from *Aspergillus niger*
- Efficient in hydrolyzing cellulose, less efficient in heterogenous substrates
- Production of *Trichoderma reesei* cellulase induced by sophorose/lactose with cellulose as carbon source
 - Cellobiohydrolase (*exo*-glucanase) constitutes about ~60% of total protein
 - Endogenous beta-glucosidase activity is low
 - GH61 proteins of unknown biochemical mechanism improves activity

Conversion of lignocellulose to fuels and products



Feedstock



Pretreatment



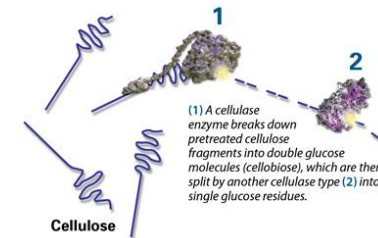
Enzyme hydrolysis



Fermentation



Fuels and products

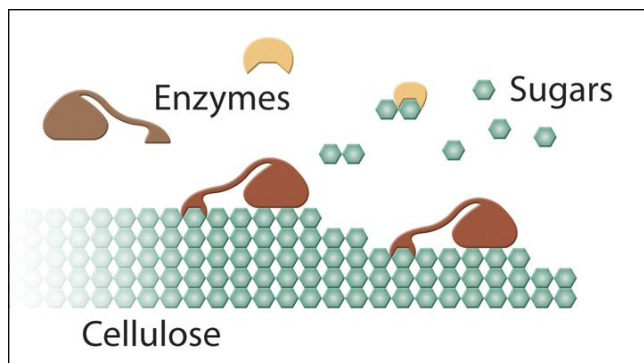




Source of biomass-degrading enzymes - microbial extracellular proteins

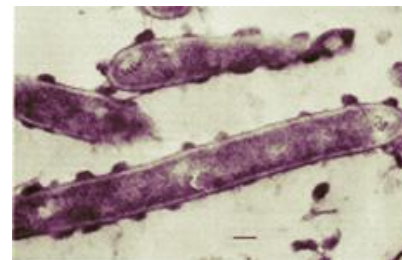
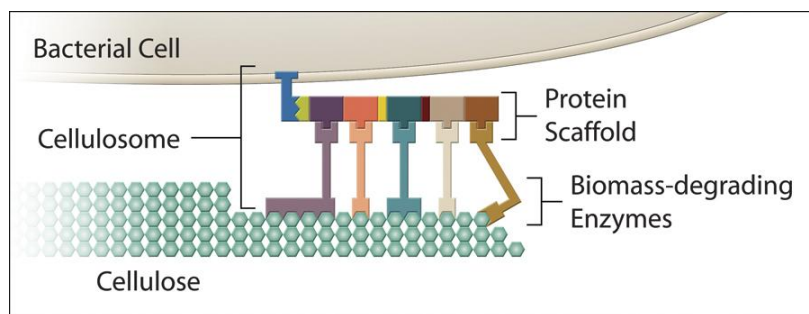
Aerobic environment (fungi)

Soluble, secreted proteins



Anaerobic environment (bacteria & fungi)

Cellulosome



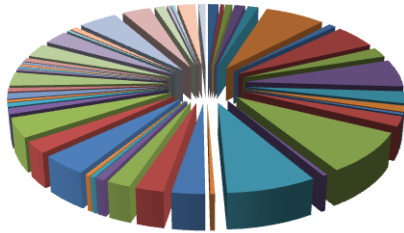


The case for diversity search for new lignocellulolytic enzymes

- *Trichoderma reesei* and *Aspergillus niger* are low-cost producers of cellulases, but they may not have the most efficient enzymes
- Efficient hydrolyzing of lignocellulose require additional enzymes, many of which may not have been characterized previously
- Diversity of the Fungal Kingdom classified
 - 7 phyla
 - 129 orders
 - Over 60,000 species
 - Fewer than 100 species been sequenced

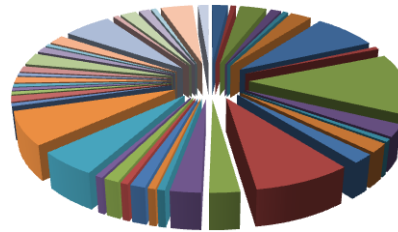


Carbohydrate-degrading enzymes (glycoside hydrolases) of select fungi



Industrial workhorse

Aspergillus niger
(Phylum: *Ascomycota*)
49 GH families
239 GH proteins



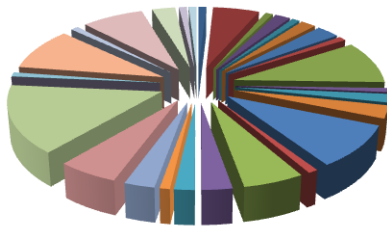
Cellulase producer

Trichoderma reesei
(Phylum: *Ascomycota*)
43 GH families
153 GH proteins



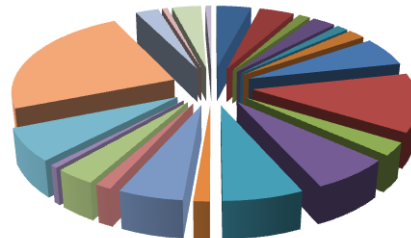
Baker's yeast

Saccharomyces cerevisiae
(*Ascomycota*)
12 GH families
19 GH proteins



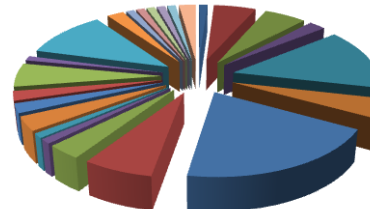
White-rot fungus

Phanerochaete chrysosporium
(Phylum: *Basidiomycota*)
29 GH families
106 GH proteins



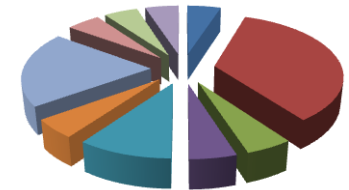
Brown-rot fungus

Postia placenta
(Phylum: *Basidiomycota*)
22 GH families
148 GH proteins



Plant pathogen

Rhizopus oryzae
(Phylum: *Zygomycota*)
24 GH families
91 GH proteins

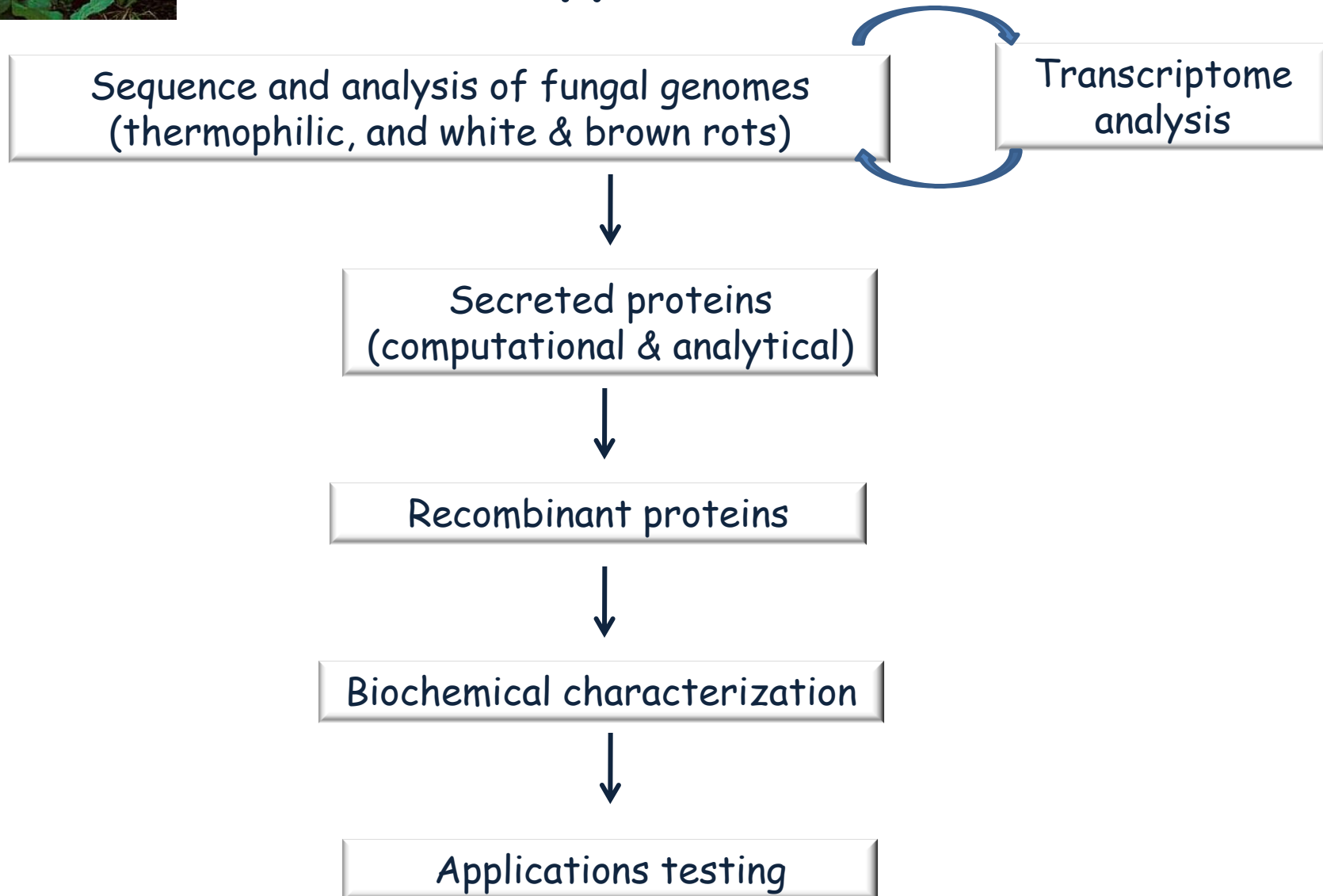


Frog pathogen

Batrachochytrium dendrobatidis
(Phylum: *Chytridiomycota*)
10 GH families
20 GH proteins

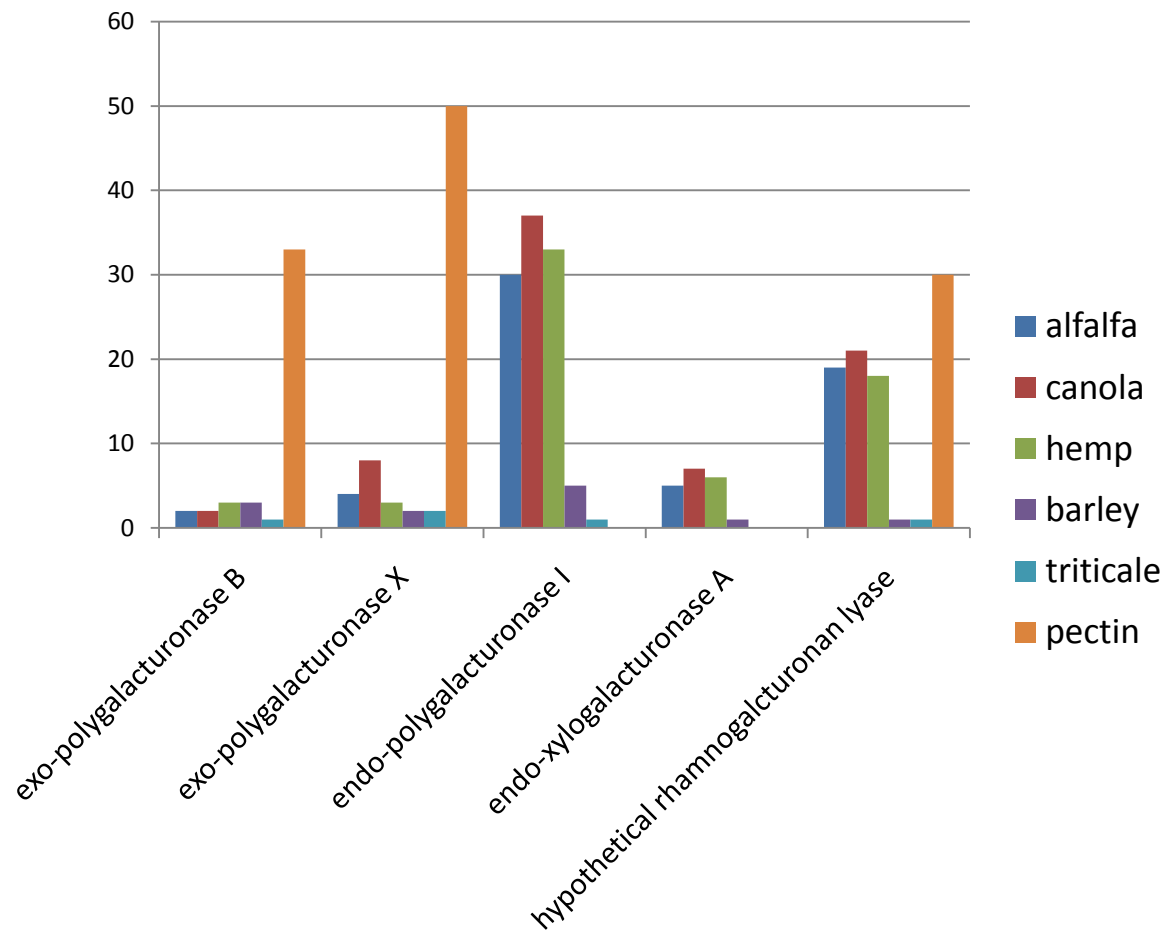


Fungal genomes to industrial applications – a vertical approach





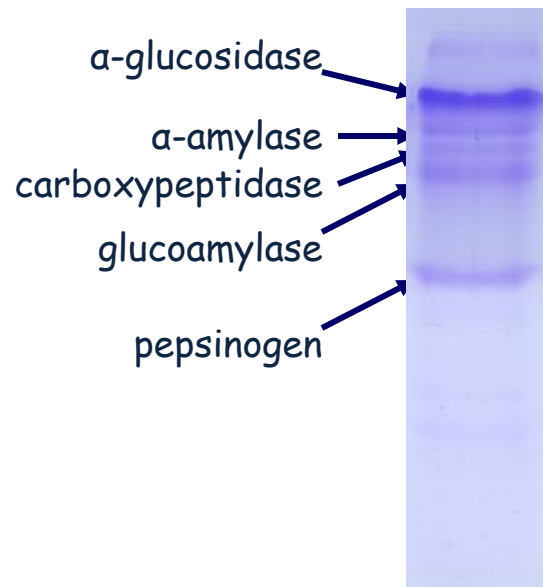
Profiling fungal lignocellulolytic enzymes induced by natural and homogeneous substrates: *exo-acting* and *endo-acting*



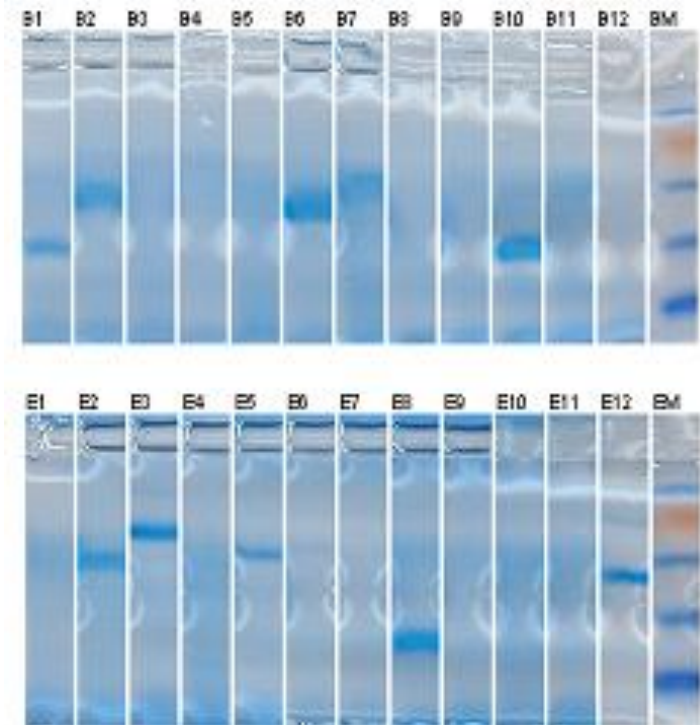


Improvement of *Aspergillus niger** as an enzyme production host

Proteins secreted in wild-type strain



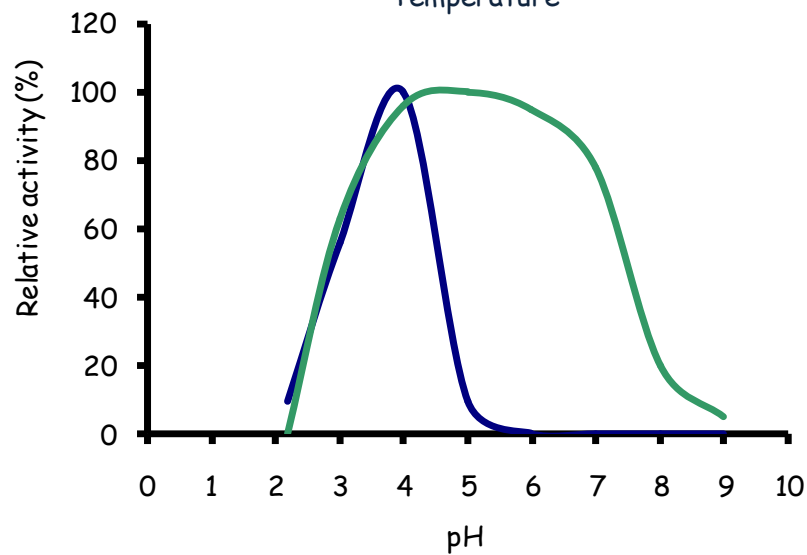
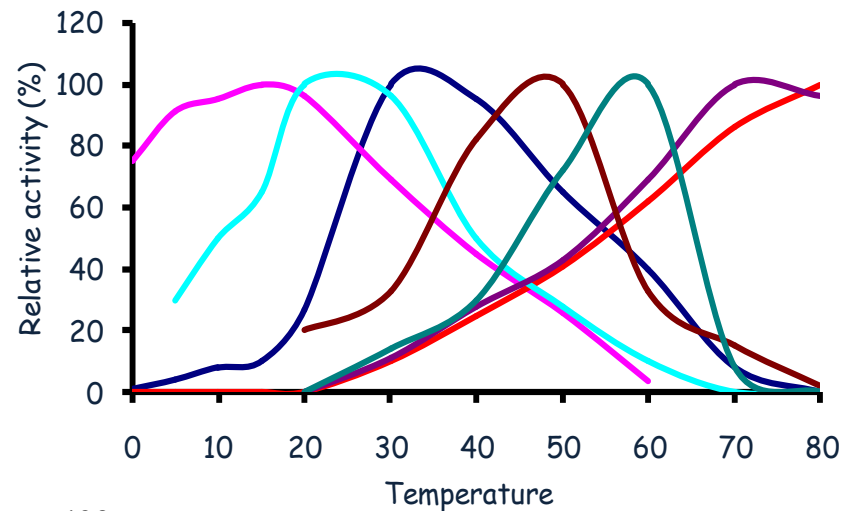
Recombinant proteins secreted in "clean" strain



*In collaboration with DSM



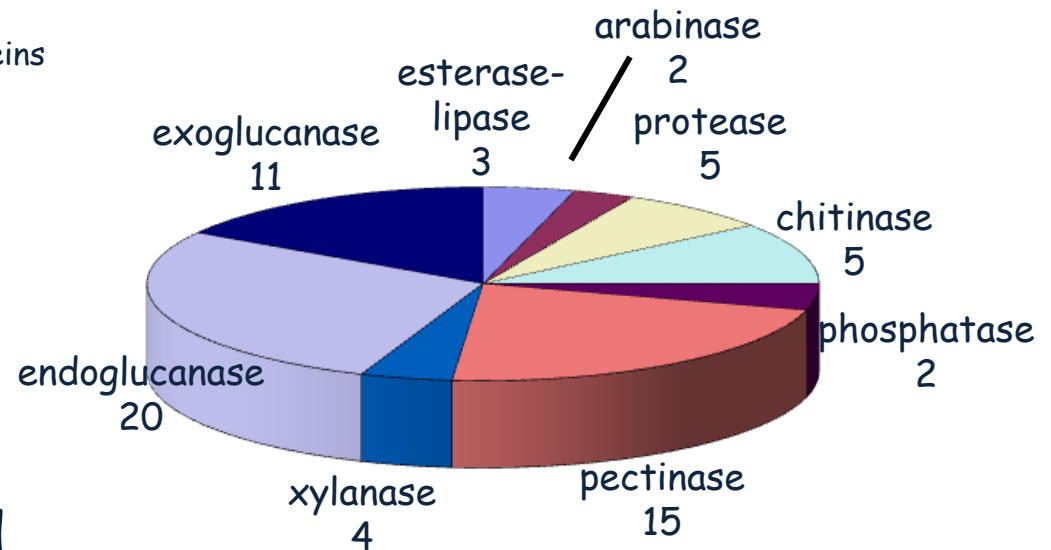
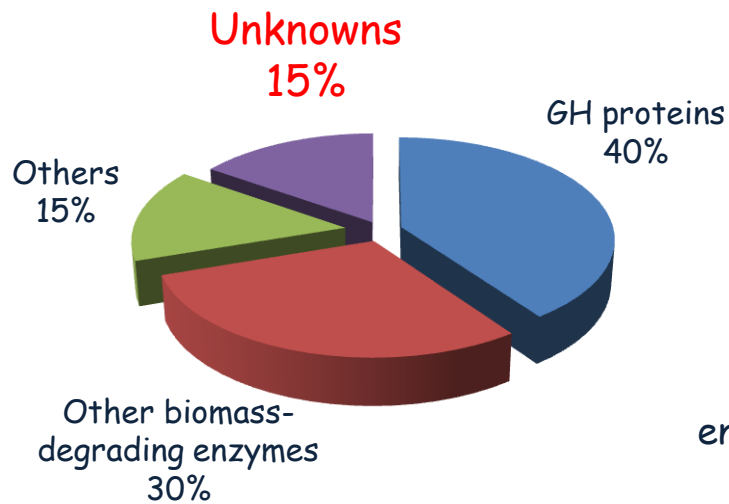
Recombinant enzymes with diverse biochemical properties





Most extracellular, unknown proteins expressed encode biomass-degrading enzymes

Recombinant hypothetical, secreted proteins are screened for activity on 36 natural and synthetic substrates



Profile of proteins secreted when cultured in complex substrates



Concluding remarks

- The search for efficient enzymes for lignocellulose degradation has just begun
- Fungi possess enormous diversity of lignocellulolytic enzymes
- Enzymes produced by fungi in the presence of homogenous substrates are very different from those induced by natural substrates
- monocot and dicot straws appear to elicit distinct profiles of lignocellulolytic enzymes



Funding sources

- Cellulosic Biofuels Network - Agricultural Bioproducts Innovation program of Agriculture & Agri-Food Canada
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Partners and collaborators



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