Enzyme production by *Trichoderma reesei* Rut C-30 followed by enzymatic hydrolysis of different lignocellulosic materials

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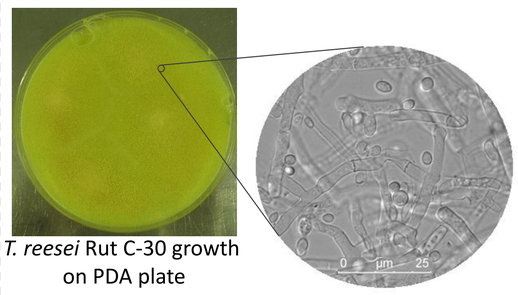
INTRODUCTION The filamentous fungus *Trichoderma reesei* is one of the main sources for cellulose degrading enzymes. We study the enzyme profile produced during the fungal growth on cellulosic and lignocellulosic substrates and their capacity to hydrolyze cellulosic and lignocellulosic substrates with different chemical and physical properties. The results will bring insight into the bottlenecks of enzymatic hydrolysis.

Trichoderma reesei



T. reesei Rut C-30 growth on PDA plate

- Soft rot fungus
- Discovered during World War II
- *T. reesei* Rut C-30 was developed by three rounds of random mutagenesis from the wild-type QM6a strain aiming for high cellulase production and catabolite derepression
- *T. reesei* Rut C-30 was developed in late 70's, but it is still considered the best producers of cellulolytic enzymes available in the public domain [1]

PRETREATMENT OF LIGNOCELLULOSE

Wood chips → Prehydrolysis → Pulp cooking → Washing → Oxygen delignification → Higly reactive cellulose

1. ANALYSIS OF PHYSICAL AND CHEMICAL PROPERTIES OF RAW MATERIAL

Cooked pulp

Oxygen delignified pulp

Sodium chlorite delignified pulp

Substrates were produced during the process of sodium hydroxide cooking, used in pulp and paper industry.

Analysis
Chemical composition: HPAEC-PAD & UV spectroscopy
Supra-molecular structure: CP/MAS 13C NMR

2. ENZYME PRODUCTION STUDY

Start of cultivation of *T. reesei* Rut C-30 on lignocellulose

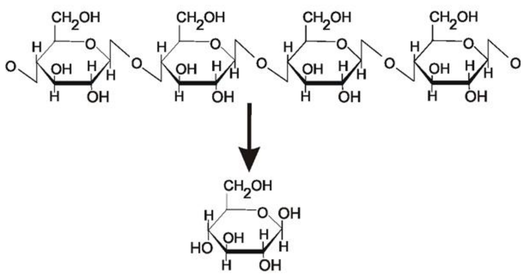
Lignocellulose hydrolysis = enzyme production

End of cultivation of *T. reesei* Rut C-30 on lignocellulose

T. reesei was grown submerged fermentations on commercial cellulose Avicel PH101 and industrial-like lignocellulosic substrates from spruce.

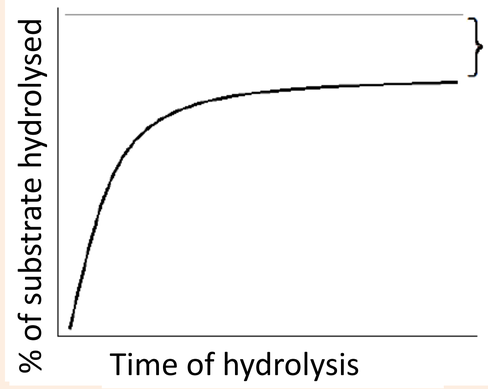
Analysis
Fungal growth: microscopy
Secreted proteins: SDS-PAGE & Bradford assay
Enzymatic activity: FPA & CMC assay

3. ENZYMATIC HYDROLYSIS



Hydrolysis efficiency of lignocellulose was compared to model cellulosic substrates, namely, Avicel PH101, nanocrystalline cellulose, phosphoric acid-swollen cellulose and cotton, which have defined characteristics.

4. STUDY OF BOTTLENECKS IN ENZYMATIC HYDROLYSIS



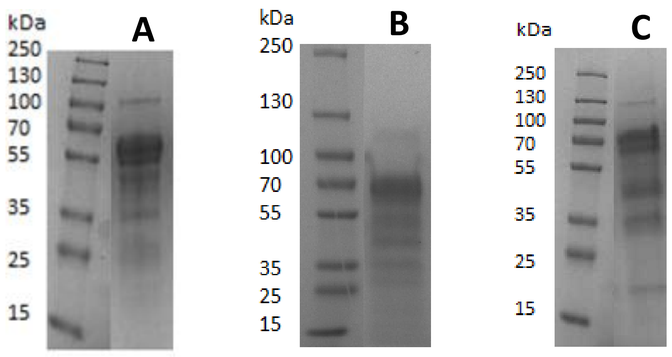
What are these structures?

Analysis
Released sugars: HPAEC-PAD
Structure of non-hydrolyzed material: CP/MAS 13C NMR
Dynamics of the hydrolysis: QCM-D

Relation of enzymatic hydrolysis data with the structural properties of the substrates will help to understand the reasons for difference in enzyme performance.

RESULTS

Enzyme profile secreted during cultivations on cellulose and lignocellulose



Summary of the protein concentrations and corresponding enzyme activities

Cultivation condition	Protein (g/l)	FPU/ml
A	3.3	3.8
B	1.1	0.8
C	0.9	0.4

FPU – filter paper units

Cultivation conditions: A – Avicel PH101; B – oxygen delignified pulp treated with sodium chlorite; C – alkaline cooked pulp

CONCLUDING REMARKS

- Filamentous fungus *T. reesei* Rut C-30 efficiently degrades cellulose and lignocellulose.
- During the growth on different cellulosic and lignocellulosic substrates the fungus secretes different profile of cellulose degradinf enzymes.
- Enzymatic hydrolysis is influenced by different chemical and physical properties of the substrates.

References

1. Peterson, R., and Nevalainen, H. (2012). Trichoderma reesei RUT-C30 - thirty years of strain improvement. Microbiology *158*, 58-68.

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