Heterologous Expression, Purification, and Characterization of a Cellobiohydrolase from *Penicillium funiculosum*

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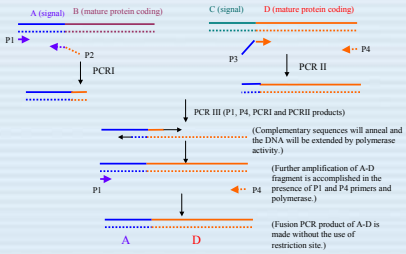
Abstract

Ascomycete and Basidiomycete fungi are recognized for their role in the biodegradation and recycling of organic matter in nature. Their ability to digest cellulosic biomass (e.g. leaf litter and wood), and in the case of many Basidiomycetes, both cellulose and lignin, is of great interest to the emerging bioenergy industry, since biomass represents an enormous renewable resource for the production of fuels and chemicals. Among the notable genera of industrially important fungi that produce Glycosyl Hydrolase family 7 cellobiohydrolases are *Trichoderma* and *Penicillium*. The cellobiohydrolases from this structural family are generally recognized as being the principal enzyme in the construction of engineered component cellulase systems designed for hydrolysis of microcrystalline cellulose. In this study, we report the heterologous expression of an active and stable full-length cellobiohydrolase from *Penicillium funiculosum* in transformed *Aspergillus awamori*. We compare the kinetics and biochemical properties of the recombinant form of the enzyme engineered and expressed using different signal sequences compared to the wild type enzyme.

Steps Used for the Production of *P. funiculosum* rCel7A

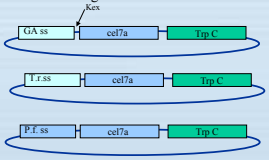
P. funiculosum ATCC62998
⇩
Isolated gene by PCR from genomic DNA
⇩
Inserted gene into fungal expression vector pFE2 using overlap extension PCR
⇩
Protoplast transformation of *A. awamori* ATCC22342
⇩
1 L shake flask growth for 4-7 days
⇩
Purification

Overlap Extension PCR



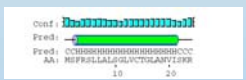
Construction of *P. funiculosum cel7a* under Different Signal Sequences

- Overlap Extension PCR
  - Signal sequence was precisely fused with mature protein coding sequence without the introduction of extra bases.
- The *E. coli* shuttle vector pFE2 was used for the cloning of the three constructs.
- GA promoter and TrpC terminator on pFE2 were used for the expression of *cel7a* gene.
- The rest of the vector sequence is common among the three constructs.



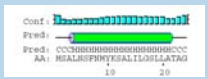
Secondary Structure Prediction of Signal Sequences

***A. awamori GA* signal - 24 aa**
m142

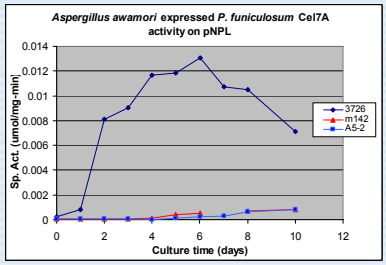


***T. reesei cel7a* signal - 17 aa**
A5-2

***P. funiculosum* signal - 24 aa**
3726

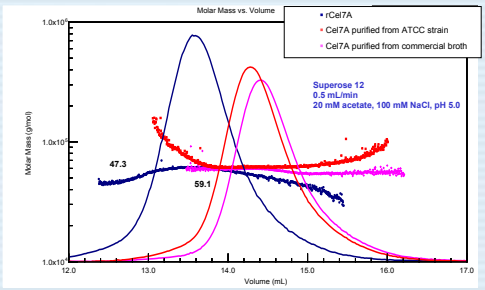


Cel7A Activity Assay of Crude Supernatants



3726: *P. funiculosum* Cel7A signal construct
m142: GA signal construct
A5-2: *T. reesei* Cel7a signal construct

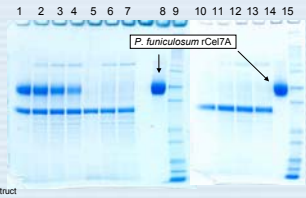
MW Comparison Using SEC-MALLS/RI of Native and Recombinant *P. funiculosum* Cel7A



SDS-PAGE Analysis of *A. awamori* Expressed *P. funiculosum* Cel7A

Crude Supernatants at Different Growth Stages

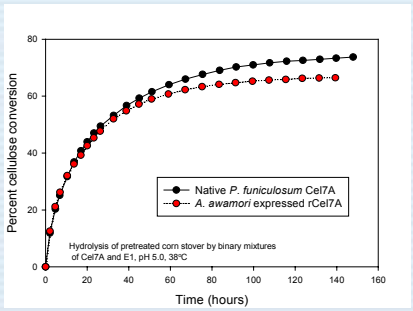
1. 3726 day 3
2. 3726 day 6
3. 3726 day 8
4. 3726 day 10
5. m142 day 3
6. m142 day 6
7. m142 day 8
8. purified 3726 rCel7A
9. Mark 12 MW Standard
10. A5-2 day 3
11. A5-2 day 6
12. A5-2 day 8
13. A5-2 day 10
14. purified 3726 rCel7A
15. Mark 12 MW Standard

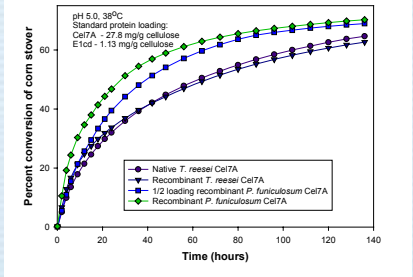


*3726: native P.f. CBHI signal construct
*m142: GA signal construct
*A5-2: T.reesei CBHI signal construct

Invitrogen NuPage 4-12% Bis-Tris, MOPS-SDS gels

Analysis of Cel7A Enzymes on Pretreated Corn Stover by Diafiltration Saccharification Assay



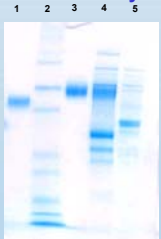


Purification of Enzymes

Culture broth
⇩
Concentrated and buffer exchange
⇩
Anion exchange chromatography
⇩
Size exclusion chromatography

SDS-PAGE Analysis of Purified rCel7 Enzymes

1. Native *P. funiculosum* Cel7A (purified)
2. Mark 12 MW standard
3. *A. awamori* expressed *P. funiculosum* Cel7A #3726 (purified)
4. *A. awamori* expressed *P. funiculosum* Cel7A m142 (semi pure)
5. *A. awamori* expressed *P .funiculosum* Cel7A A5-2 (purified)



*3726: native *P.funiculosum* signal construct
m142: GA signal construct
A5-2: *T.reesei cel7a* signal construct

Invitrogen NuPage 4-12% Bis-Tris, MOPS-SDS gels

Conclusions

- *Penicillium funiculosum* Cel7A was cloned using PCR and expressed under three different signal sequences in *Aspergillus awamori*. The Cel7A with the native signal sequence exhibited the highest specific activity.
- HPLC/MALLS analysis of native and recombinant *P. funiculosum* Cel7A showed homogeneity with respect to molecular weight and R_{rms} across the elution profile.
- Diafiltration Saccharification Assay (DSA) showed that *A. awamori* expressed and native *P. funiculosum* Cel7A performed similarly for the cellulose conversion.
- With ½ loading in DSA, *A. awamori* expressed rCel7A performed significantly better than full loading of *T. reesei* Cel7A on pretreated corn stover.

Acknowledgement

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