Comparative Thermodynamic Characterization of Cutinases Synthesized in *Pichia pastoris*

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Abstract

Cutinases are α/β hydrolases commonly secreted by fungal phytopathogens that enable pathogens to penetrate the protective cutin layer of plant, causing its destruction. To date, the *Fusarium solani f. pisi* cutinase has been thoroughly investigated for structure-function analysis. A number of other cutinases have been functionally investigated, however, such proteins have not been structurally characterized. The known crystal structures of these enzymes demonstrate several unique structural arrangements, which engender different potential applications than lipases. Here we describe the characterization of sequence-structure-activity of a set of cutinases from from *Alternaria brassicicola* (AbC), *Aspergillus fumigatus* (AfC), *Aspergillus oryzae* (AoC), *Humicola insolens* (HiC) and compare it to the well-studied *F. solani* (Fsc) cutinase.

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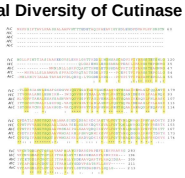
Searching Natural Diversity of Cutinase

- **Database**
  PDB and Genebank
  FsC sequence as parental sequence
- **Selection of target cutinase sequences**
  - select the sequences according to homology
  - 116 possible cutinase sequences were identified
- **Eliminating mutants of the certain parent enzymes as well as enzymes previously studied.**



Six enzymes that exhibited a sequence identity that was between 45%-65% over a minimum overlap of 175 amino acids were selected.

No reports about assessing the activity of these six cutinases, just amino acid sequences were published in the Genebank database.

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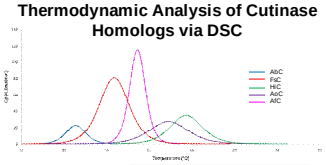
Thermodynamic Analysis of Cutinase Homologs via DSC



Assay Conditions
Protein Concentration 1 mg/ml
20 mM sodium phosphate pH 8.0
Scan Rate 1°C/min

Enzyme	ΔH KJ/mol	ΔS KJ/mol/K	ΔG Kcal/mol *
AbC	380.24	1.17	-7.50
FsC	574.17	1.76	-11.80
HiC	519.98	1.55	-13.62
AoC	562.62	1.73	-11.21
AfC	695.89	2.07	-18.6

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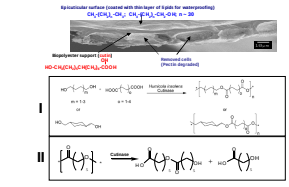
Cutinase Mediated Reactions (Natural and Synthetic)



Pio, T. Adv. In Apply. Micro. (2009)
Hunsen, M. Macromolecules (2007)

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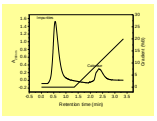
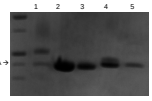
Purification of Cutinase Homologs



Column: POROS MC 16mmD100mmL
Buffer A: 50 mM NaH_2PO_4 pH 8.0
Buffer B: Buffer A and 500 mM imidazole
Elute gradient: 3CV, 0-20%B
Flow rate: 30ml/min



1. AbC
2. FsC
3. AoC
4. AfC
5. HiC

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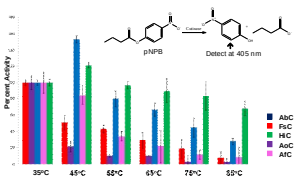
Residual Activity for pNPB



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Crystal Structure of Cutinase

- Pivotal role in the penetration of intact plant surfaces
- Hydrolysis of Cutin
  - ω-hydroxy fatty acids
  - dihydroxy palmitic acid
  - 18-hydroxy-9,10,-epoxy C18 saturated fatty acid
  - 9,10,18-trihydroxy C18 unsaturated fatty acid
- Member of serine hydrolase family
- Catalytic triad composed of Ser, His and a carboxyl group
- Oxyanion hole Ser, Gln; which stabilize the transition state

pdb code: 1cex
Longhi, S. J. Mol. Biol (1997)
Pio, T. Adv. In Appl. Micro. (2009)
Kolattukudy, P.E Arch. Biochem. Biophys (1975)

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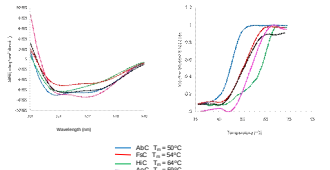
Circular Dichroism Analysis



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Conclusion

- **We successfully synthesized and purified 4 new cutinase homologs**
- **All of the homologs have a similar secondary structure**
- **In terms of T_m HiC > AoC > AfC > FsC > AbC**
- **In terms of residual activity HiC > AbC > AfC > FsC > AoC**
- **The HiC exhibits the highest thermostability and residual activity when compared to the other cutinases**

Future Direction

- **Determine thermodynamic parameters as a function of pH**
- **Explore the activity of these 'new' cutinase towards both natural and synthetic polymers**

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Acknowledgements

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