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A novel xyloglucanase from the white rot fungus *Abortiporus biennis* and its potential role as an accessory biocatalyst in the enzymatic degradation of xyloglucan-containing substrates

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INTRODUCTION & AIM

Lignocellulosic biomass is a composite material consisting of cellulose, hemicellulose, and lignin. Usually, cellulose fibrils are covered with ***xyloglucan***, a complex, highly substituted plant biomass hemicellulose. Xyloglucan is present in many plant species as a seed storage polysaccharide or as a component of the primary cell wall.

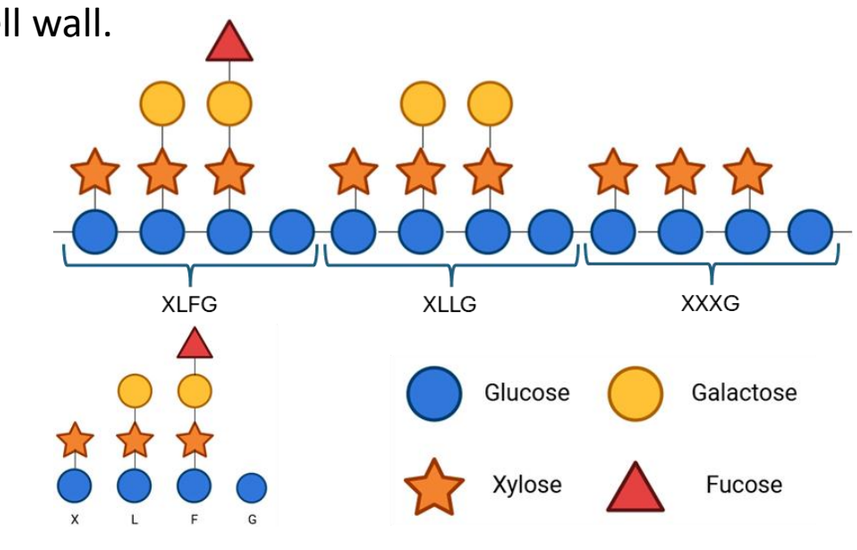

Figure 1: Primary structure of xyloglucan

Xyloglucan requires many enzymes with complementary specificities for its complete breakdown called ***xyloglucanases*** (XEGs, EC 3.2.1.151). They hydrolyze backbone β-1,4-glucosidic bonds, releasing shorter oligomers. In this study, we characterized ***Abi*Xega**, a novel XEG from the white-rot fungus *Abortiporus biennis*, known for its lignocellulose-degrading capabilities.

The need for xyloglucan removal is evident in biorefinery applications, where platform sugars must be obtained for the valorization of cellulosic biomass, but also in the improvement of animal feed, such as corn bran and apple pulp.

METHOD

Expression and purification of *Abi*Xega

E.coli Top 10 transformation → Plasmid isolation & linearization → Electroporation & Expression in *P.pastoris* X33 → Filtration of culture supernatants → Purification with IMAC

- Bioinformatics analysis
- Biochemical characterization
- Mode of action studies using DNS method to detect reducing sugars
- Synergism with commercial cellulases

RESULTS & DISCUSSION

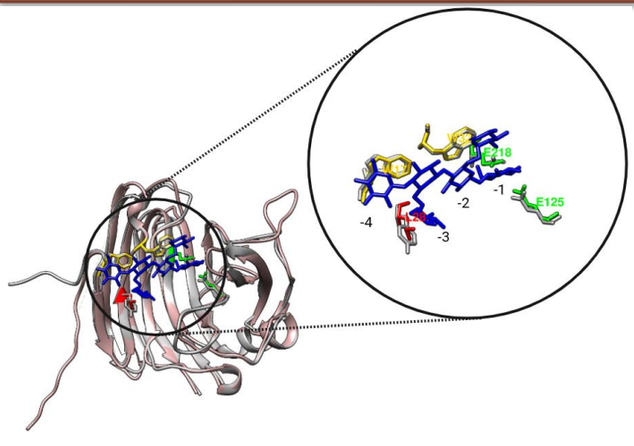

Figure 2: Superimposition of the Alphafold model structure of *Abi*Xega (pink) with *Aspergillus aculeatus* XEG (grey) and close-up view of the ligand binding site. (Ligand GXXG: blue, catalytic glutamates of *Abi*Xega green, Leu28: red, Trp17 and Trp32: yellow)

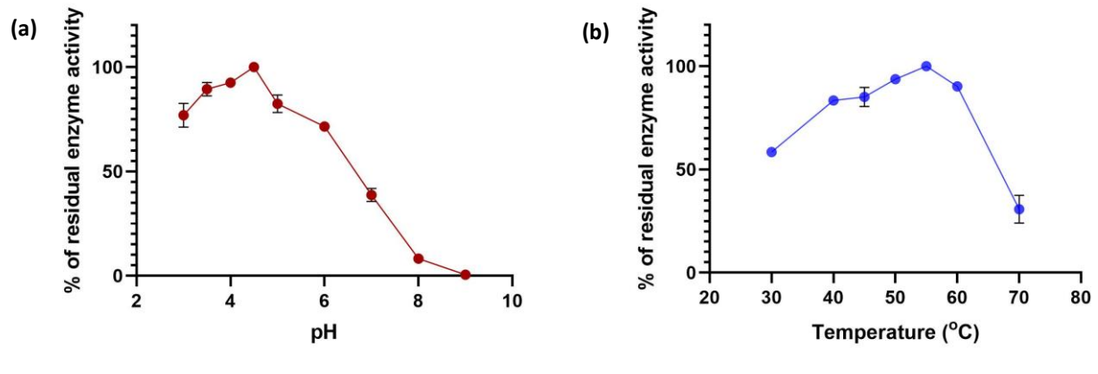

Figure 3: Optimum activity conditions of *Abi*Xega. Effect of (a) pH and (b) temperature on the activity of *Abi*Xega during xyloglucan hydrolysis.

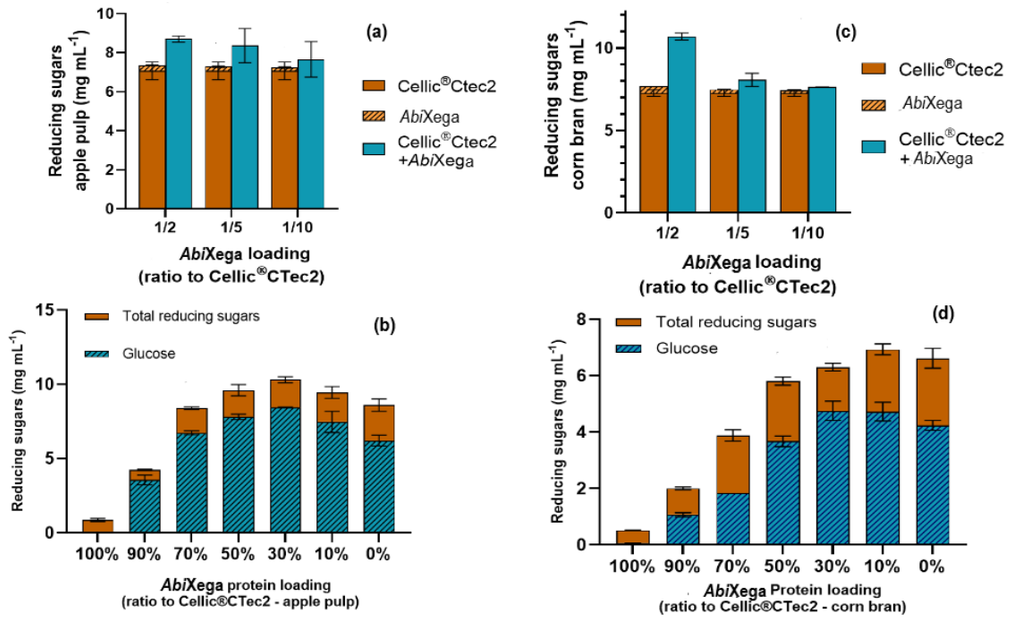

Figure 4: Synergism of *Abi*Xega and cellulases in apple pulp (a, b) and corn bran (c, d) hydrolysis. Effect of *Abi*Xega protein loading on the reducing sugars release from (a) apple pulp and (c) corn bran. (b), (d) Effect of *Abi*Xega dosage on a total protein content of fixed protein loading on the production of reducing sugars and glucose by cellulases.

CONCLUSION

- Detectable activity in xyloglucan and β-glucan
- Enhances the action of cellulases in corn bran and apple pulp
- Reaction's total protein loading minimized, without loss in reducing sugars release → lower enzyme costs for substrates with a high xyloglucan content

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