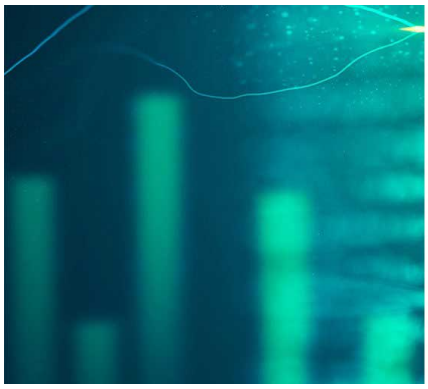
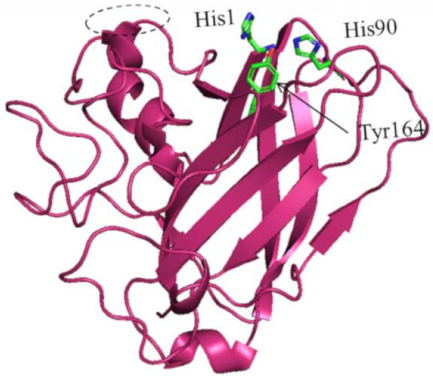
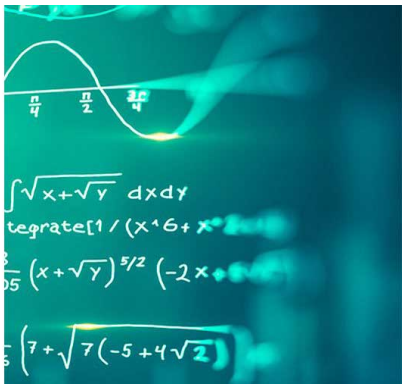






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3 minutes of reading

News

Fundamental Research

Renewable energies | Biofuels and e-fuels

Biosciences and biotechnologies | Fermentation

To optimize the breakdown of biomass required for bioethanol production, a research thesis conducted at IFPEN focused on studying new enzymes derived from fungal strains belonging to the genus *Aspergillus*. The research identified a new family of enzymes able to increase cellulase activity.

For a more efficient *Trichoderma reesei* enzyme cocktail

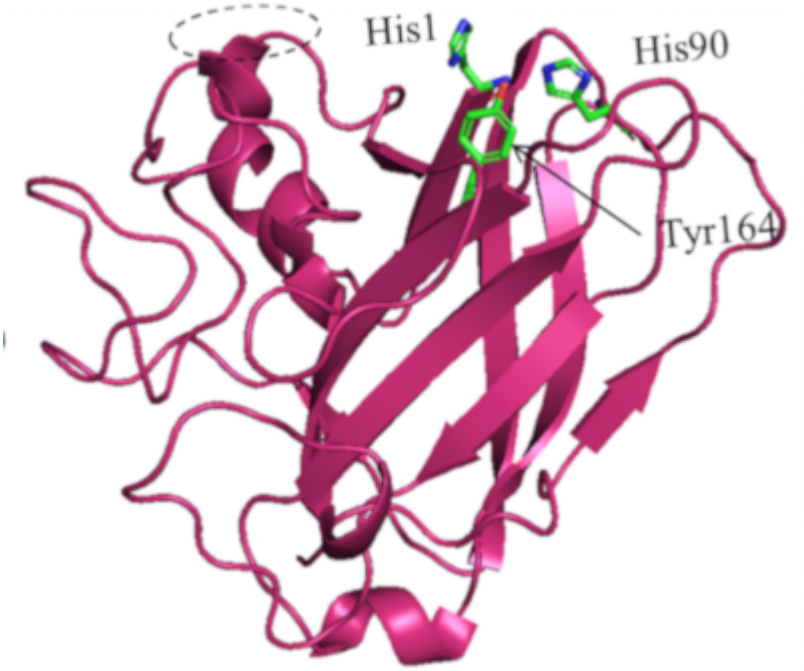
Lignocellulosic biomass is an abundant and renewable alternative to fossil resources, but its efficient break-down is a major challenge for the development of a low-cost bioethanol process.

This challenge was the focus of a PhD thesis [1], conducted in partnership with INRAE's "Biodiversité et Biotechnologie Fongiques" (BBF - Fungal Biodiversity and Biotechnology) laboratory in Marseille. The aim was to search for new enzymes able to increase the efficiency of the *Trichoderma reesei* enzyme cocktail, which is already used in second-generation ethanol production processes for the breakdown of biomass prior to fermentation.

Complementary enzymes in *Aspergillus* strains

In a previous ANR (E-TRICEL[1]) project, fungal strains belonging to the genus *Aspergillus* and producing enzymes that are complementary to *T. reesei* cellulases had been identified. Following on from that study, the “secretomes” (= the whole set of secreted enzymes)) of 5 *Aspergillus* strains were studied for their capacity to improve the activity of the reference cocktail.

Two of these secretomes, produced by an *Aspergillus japonicus* strain, showed the capacity to significantly enhance the hydrolysis of a biomass of interest, Miscanthus grass. They contained a protein with structural similarities to LPMOs (lytic polysaccharide monooxygenases[2]), in particular the presence of conserved Histidine[3] residues that typically coordinate a Cu^{2+} ion (diagram).



3D model of A. Japonicus AA16. The amino acids involved in the coordination of Cu2+, essential for catalytic activity, are shown in green.

Enzyme tests then confirmed that this protein possessed an oxidative activity and was able to improve the activity of the *T. reesei* CBH1 cellulase on cellulosic substrates [2].

AA16: a new family to be characterized

Since these new enzymes have a structure that differs from those of known LPMOs, they represent a new family in the CAZy database (www.cazy.org) classifying all carbohydrate-active enzymes.

Subsequently, several other members of this new family, named AA16, demonstrated the same capacity to release oxidized sugars and increase cellulase activity.

In-depth characterization of AA16 enzymes will be the focus of another PhD thesis. In particular, the objective will be to determine the identity of optimal redox partners, as well as their three-dimensional

structure, via crystallography, and to study the synergistic activity with cellulases on real lignocellulosic substrates.

[1] Entitled *Exploration de la biodiversité enzymatique pour la complémentation du secrétome de* Trichoderma reesei *afin d'améliorer l'hydrolyse des lignocelluloses* (Exploration of enzyme biodiversity for complementation of the Trichoderma reesei secretome in order to improve lignocellulosic hydrolysis)
[2] Enzymes that cleave polysaccharides via an oxidative mechanism
[3] One of the amino acids constituting proteins

References :

[1] Camille Filiatrault-Chastel's thesis: "Exploration de sécrétomes d'*Aspergillus* spp. en vue d'une complémentation du cocktail cellulolytique de *Trichoderma reesei*" (Exploration of *Aspergillus* spp secretomes, for the complementation of the *Trichoderma reesei* cellulolytic cocktail), 2019.
[2] Filiatrault-Chastel, C., Navarro, D., Haon, M., Grisel, S., Herpoël-Gimbert, I., Chevret, D., Fanuel, M., Henrissat, B., Heiss-Blanquet, S., Margeot, A., and Berrin, J.-G. AA16, a new lytic polysaccharide monooxygenase family identified in fungal secretomes. Biotechnol Biofuels 12, 55 (2019), DOI: 10.1186/s13068-019-1394-y

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Boosting cellulase activity to reduce the cost of bioethanol
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