Post-doctoral position 24 months

Understanding the mode of action of LPMOs and expansins

INRAE Biopolymers, Interactions and Assemblies (BIA)

INRAE Fungal Biodiversity and Biotechnology (BBF)

University of Toronto (UoT)

Contract start: Spring 2025

Employer: INRAE

Supervision:

- INRAE Biopolymers, Interactions and Assemblies (BIA) in Nantes (France): by Ana Villares and Céline Moreau (https://eng-ur-bia.angers-nantes.hub.inrae.fr/)
- INRAE Fungal Biodiversity and Biotechnology (BBF) lab in Marseille (France): by Jean-Guy Berrin (https://www.bbf-lab.fr/page/Home)
- University of Toronto (Canada): by Emma Master (https://www.labs.chem-eng.utoronto.ca/master/)

The postdoctoral fellowship will be mainly performed in Nantes (France), with internships at BBF laboratory (Marseille, France) and at the University of Toronto (Canada).

Scientific context and project summary

This postdoctoral position is part of the SmartCoupling project, funded by the PEPR B-Best (France 2030), which aims to combine enzymes and chemistry to create sustainable and tailored tools for lignocellulosic biomass (LCB) fractionation and conversion into functionalized building blocks.

LCB can be viewed as a promising sustainable source of energy and materials. For this reason, its degradation has attracted much interest for the development of biofuels as well as chemicals and nanocelluloses.[1,2] The major bottleneck of lignocellulose utilization is its inherent recalcitrance, arising from the structural complexity leading to semi-crystalline arrangement of polysaccharides.[3] For this reason, biomass fractionation makes necessary the addition of pretreatments including chemical, mechanical and/or enzymatic methods, which increase costs (energy) and environmental footprint (chemicals, water, waste). While humankind tries to overcome biomass recalcitrance in a sustainable way, nature has already set it up by a battery of enzymes evolved by microorganisms for cell wall degradation. In this field, fungi secrete a number of enzymes, whose synergistic activity achieves lignocellulose degradation. Among these enzymes, lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that oxidatively cleave polysaccharides.[4] LPMOs have already demonstrated a key role on cellulose fractionation.[2] Thus, BIA and BBF have described for the first time the effect of LPMOs on the cellulose fiber, and we demonstrated that LPMOs attack the tension zones of the fiber and create nicking points, which facilitates fibrillation.[1,5] Although LPMO action on the cellulose fiber is promising, LCB is a much more complex substrate that may hinder their enzymatic action.

SmartCoupling will focus on LPMOs as oxidative enzymes, and the use of expansins to disrupt the fiber structure in a controlled way. Expansin-related proteins have demonstrated to disrupt non-covalent bonds between cellulose, and/or between cellulose and polysaccharides including hemicelluloses and

pectin.[6] Therefore, expansins have potential to increase fiber swelling and accessibility, which could boost the LPMO action and further chemical functionalization to obtain cellulose nanofibers by applying mild mechanical treatments.

Research program

This postdoctoral position aims at investigating the action of LPMOs and expansins on the LCB. The purpose of the study is to control the enzymatic activity for two main goals: (i) to get more insight into the mechanism of action of LPMOs and expansins, and (ii) to use both as a tool to fractionate LCB. For this purpose, we propose the following objectives:

1. To design model substrates to study the enzymatic action on the different components of LCB.
2. To understand the mechanism of action of LPMOs and expansins on different LCB substrates.
3. To define enzyme-assisted protocols for LCB fractionation.

Candidate profile

We look for a candidate with initiative, creativity and team working ability, with a PhD in Chemistry, Physical Chemistry, Biochemistry or related. Expertise in lignocellulosic biomass will be highly considered, as well as in common characterization techniques in Physics and Nanotechnology (FTIR, UV-vis spectroscopy, NMR, EA) as well as specific techniques for the study of biopolymers (microscopy, AFM, TEM, Conductometry, DLS, X-Ray, birefringence, etc.). Knowledge of at least one of the following topics is particularly welcome: biopolymers, physical chemistry of biopolymers, nanotechnology, enzymes.

Application procedure

Send a brief CV (maximum 2 pages) and a cover letter to Ana Villares (ana.villares@inrae.fr), Emma Master (emma.master@utoronto.ca) and Jean-Guy Berrin (jean-guy.berrin@inrae.fr) including two references for possible recommendation.

Deadline for applications: February 16.

References

1. Villares, A.; Moreau, C.; Bennati-Granier, C.; Garajova, S.; Foucat, L.; Falourd, X.; Saake, B.; Berrin, J.G.; Cathala, B. Lytic polysaccharide monooxygenases disrupt the cellulose fibers structure. *Sci Rep* **2017**, *7*, 40262, doi:DOI: 10.1038/srep40262.
2. Moreau, C.; Tapin-Lingua, S.; Grisel, S.; Gimbert, I.; Le Gall, S.; Meyer, V.; Petit-Conil, M.; Berrin, J.G.; Cathala, B.; Villares, A. Lytic polysaccharide monooxygenases (LPMOs) facilitate cellulose nanofibrils production. *Biotechnol. Biofuels* **2019**, *12*, doi:10.1186/s13068-019-1501-0.
3. Marriott, P.E.; Gómez, L.D.; McQueen-Mason, S.J. Unlocking the potential of lignocellulosic biomass through plant science. *New Phytologist* **2016**, *209*, 1366-1381, doi:https://doi.org/10.1111/nph.13684.
4. Bissaro, B.; Eijsink, V.G.H. Lytic polysaccharide monooxygenases: enzymes for controlled and site-specific Fenton-like chemistry. *Essays in biochemistry* **2023**, 10.1042/ebc20220250, doi:10.1042/ebc20220250.
5. Chemin, M.; Kansou, K.; Cahier, K.; Grellier, M.; Grisel, S.; Novales, B.; Moreau, C.; Villares, A.; Berrin, J.G.; Cathala, B. Optimized Lytic Polysaccharide Monooxygenase Action Increases Fiber Accessibility and Fibrillation by Releasing Tension Stress in Cellulose Cotton Fibers. *Biomacromolecules* **2023**, *24*, 3246–3255, doi:10.1021/acs.biomac.3c00303.
6. McQueen-Mason, S.; Cosgrove, D.J. Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. *Proceedings of the National Academy of Sciences* **1994**, *91*, 6574-6578, doi:doi:10.1073/pnas.91.14.6574.

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