UTILIZING A LYTIC POLYSACCHARIDE MONOOXYGENASE FROM *FUSARIUM OXYSPORUM* FOR THE ADVANCED PRODUCTION OF NANOCELLULOSE FROM WHEAT STRAW

K. Chorozian[1], A. Karnaouri[2], M. Ponjavic[3], M. Dimarogona[4], J. Nikodinovic[3], E. Topakas[1]*

[1] InduBioCat Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

[2] Laboratory of General and Agricultural Microbiology, Department of Crop Science, Agricultural University of Athens, Athens, Greece

[3] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

[4] Laboratory of Structural Biology and Biotechnology, Department of Chemical Engineering University of Patras, Patras, Greece

(*vtopakas@chemeng.ntua.gr)

ABSTRACT

The focus of this study is on the characterization of different lytic polysaccharide monooxygenases (LPMOs) belonging to the AA9 family of CAZy database (http://www.cazy.org/), aiming to explore their potential in enhancing the production of bio-based materials. A recently identified and characterized AA9 LPMO from *Fusarium oxysporum* (*Fo*LPMO9) is investigated, with an emphasis on harnessing its capabilities for the isolation of nanocellulose, a promising green biomaterial with a great potential to replace plastics [1]. *Fo*LPMO9 was expressed heterologously in *Pichia pastoris* under the methanol-induced AOX1 promoter, followed by biochemical and functional characterization. Notably, *Fo*LPMO9 exhibited versatile regioselectivity, demonstrating C1, C4, and C1/C4 oxidative cleavage patterns on cellulose substrates. At the next step, an enzymatic process for the isolation of nanocellulose from the solid pulp of OxiOrganosolv pretreated wheat straw biomass[2] was introduced. The process entailed a multi-step enzymatic treatment utilizing hemicellulases and cellulases[3], succeeded by the oxidative cleavage mediated by LPMOs. Apart from *Fo*LPMO9, other LPMOs with different stereoselectivity, namely *Tth*LPMO9G[2] and *Mt*LPMO9H[4], were tested. Moreover, a post-treatment step with each AA9 LPMO was also conducted in order to assess their varied effects on the final nanocellulose product. The efficacy of this approach was validated through advanced nanocellulose characterization techniques. The findings underscore the significance of employing AA9 LPMOs for the enzyme-mediated isolation, as well as the functionalization of nanocellulose, providing a sustainable and efficient approach for the production of bio-based, high-added-value products from lignocellulose.

KEYWORDS: Nanocellulose, Lytic Polysaccharide Monooxygenases, *Fusarium oxysporum*, Wheat Straw

REFERENCES

[1] Karnaouri A, Chorozian K, Zouraris D, Karantonis A, *et al.* (2022) *Bioresour Technol.* 345, 126491.
[2] Kalogiannis KG, Karnaouri A, Michailof C, Tzika AM, et al. (2020) *Bioresour Technol.* 313, 123599.
[3] Chorozian K, Karnaouri A, Karantonis A, Souli M, Topakas E. (2022) *ACS Sustain Chem Eng*. 10(27), 8919.
[4] Karnaouri A, Jalvo B, Moritz P, Matsakas L, Rova U, *et al.* (2020), *ACS Sustain Chem Eng*. 8, 50.