

Production of furan-based renewable monomers by employing novel enzymes of fungal origin

Evangelia-Loukia Giouroukou¹, Maria-Konstantina Karonidi¹, Koar Choroizian², Grigorios Dedes², Georgios I. Zervakis¹, Anthi Karnaouri¹, Evangelos Topakas^{2*}

¹ *Laboratory of General and Agricultural Microbiology, Department of Crop Science, Agricultural University of Athens*

² *Industrial Biotechnology & Biocatalysis Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens*

* vtopakas@chemeng.ntua.gr

Plastics have become ubiquitous materials, present in almost every aspect of modern life, however their dependence on dwindling fossil fuel sources and their durability has raised concerns about their sustainability and the impact of their use in the environment. The development of biobased polymers, including biobased and/or biodegradable plastics, addresses the need for greener, environmentally responsible materials. Furans, such as 5-(hydroxymethyl)furfural (HMF) and furfural (FA), that can be obtained by chemical dehydration of sugars, have gained significant attention due to their chemical attributes as platform chemicals for the production of bio-polyester materials [1]. HMF is the precursor of 2,5-furandicarboxylic acid (FDCA), a monomer of polyethylene 2,5-furandicarboxylate (PEF) which is an alternative to polyethylene terephthalate (PET) produced from fossil fuel, while 2-furancarboxylic acid (FCA), produced by FA oxidation, also possesses significant potential for use as a monomer in polyesters. Biocatalytic oxidation of furans with redox enzymes offers a facile and regioselective route of reaction under mild conditions [2]. The current study targeted at the enzymatic biotransformation of HMF and FA using novel fungal biocatalysts from the Auxiliary Activity AA3 and AA5 families of CAZy database [3]. Through intelligent exploration of *Ganoderma lucidum* genome, it was possible to retrieve one sequence with putative glyoxal oxidase activity (*GI*GlyOx1) and one with aryl-alcohol oxidase (*GIA*AOx1) activity based on their homology with known furan-transforming fungal catalytic activities. The genes were heterologously expressed in yeast *Pichia pastoris*, the respective enzymes were purified to their homogeneity and biochemically characterized. *GI*GlyOx1 and *GIA*AOx1 were evaluated, both individually and synergistically (along with the presence of *in-house* produced galactose oxidase *FoGalOx* from *Fusarium oxysporum* [4] and a commercially available horseradish peroxidase HRP), for their ability to act on furans and produce value-added oxidized derivatives. Our results demonstrate the potential of *G. lucidum* enzymes for obtaining furan-based monomers from lignocellulosic biomass residues, which can be used as building blocks for the production of biobased polymers.

Keywords: Biotransformation, Enzyme catalysis, Oxidases, CAZymes, Furans, Basidiomycete

Acknowledgements: The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “3rd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers” (Project Number: 7315).

References

- [1] van Putten R-J, et al. **2013**, *Chem. Rev.* 113, 3, 1499–1597
- [2] Cajnko M. et al. **2020**, *Biotechnol Biofuels* 13, 66
- [3] Drula E. et al. **2022**, *Nucleic Acids Res* 50: D571–D577
- [4] Dedes G., Karnaouri A., Topakas E. **2022**, P-234, 13th PESXM, Patras, Greece