Enzyme-mediated transformation of lignocellulose-derived 5-hydroxymethylfurfural to the precursor for polymer synthesis 2,5-furandicarboxylic acid

Anthi Karnaouri[1*], **Maria-Konstantina Karonidi[1]**, **Evangelia-Loukia Giouroukou[1]**, **Koar Chorozian[2]**, **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*

E-mail: akarnaouri@aua.gr

Keywords: biocatalysis, oxidase, furans, HMF, FDCA

Furans, including 5-hydroxymethylfurfural (HMF) and its oxidized derivative 2,5-furandicarboxylic acid (FDCA), are promising compounds that can be used as precursors for polymer synthesis due to their high chemical reactivity. Producing these compounds from sugar streams derived from lignocellulose presents a promising alternative to fossil fuels, allowing for the conversion of biomass into higher-value chemicals [1]. This study focuses on exploiting a mild and regioselective biocatalytic process using novel fungal enzymes for the biotransformation of HMF and its oxidative derivatives into valuable monomers like FDCA. Two specific enzymes, one with glyoxal oxidase activity (*Gl*GlyOx) and another with galactose oxidase activity (*Fo*GalOx), were identified from the Auxiliary Activity AA5 family (AA5) of the CAZy database, while an aryl alcohol oxidase (*Gl*AAO) from the AA3 family was also selected for further study. These genes were expressed heterologously in the methylotrophic yeast *Pichia pastoris*, and the enzymes were purified and characterized biochemically. The enzymes were tested for their ability to transform furans into oxidized derivatives, both individually and in combination with horseradish peroxidase (HRP), as well as *in-house* produced fungal unspecific peroxygenases (UPOs). *Fo*GalOx was found to catalyze the conversion of various furanic compounds, while *Gl*GlyOx specifically oxidized HMF to 5-hydroxymethyl-2-furancarboxylic acid (HMFCA) and furan-2,5-dicarbaldehyde (DFF) to 5-formylfurancarboxylic acid (FFCA). *Gl*AA efficiently oxidized HMF to FDCA, while the presence of HRP and UPOs significantly enhanced these reactions. The addition of catalase, which consumed excess hydrogen peroxide, further improved the process by preventing adverse effects from its accumulation. Overall, the results highlight the potential of these enzymes to oxidize furan substrates from lignocellulosic biomass, producing high-value compounds with diverse industrial applications.

[1] Dedes A. et al., “Novel routes in transformation of lignocellulosic biomass to furan platform chemicals: from pretreatment to enzyme catalysis”, Catalysts, vol. 10, no. 7, pp. 743, 2020.