BIOTRANS
LA ROCHELLE 2023

N°1372 / PC
TOPIC(s) : Enzyme discovery and engineering

# ALKALI- AND HYPERTHERMO-STABLE ENGINEERED LACCASE FOR LIGNIN-RELATED PHENOLICS.

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## PURPOSE OF THE ABSTRACT

Laccases catalyze the oxidation of various substrates, aromatic amines, phenols, and metal ions, coupled with the reduction of molecular oxygen to water. Laccases have a remarkable redox ability and are in the front line for establishing enzymatic bioconversions of aromatics [1]. We have previously reported a laboratory evolution approach that led to the improved efficiency of the Aquifex aeolicus McoA hyperthermophile bacterial metallo-oxidase for the typical laccase substrate ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)) while showing an enhanced kinetic and thermodynamic thermostability [2]. In this study, we have used a combination of directed evolution (DNA-shuffling and error-prone PCR followed by high-throughput screening) and rational design to evolve the McoA 2B3 variant for 2,6-dimethoxyphenol (syringol), a lignin-related phenolic substrate. These experiments led to the identification of the hit variant, McoA 15E6-ΔMetloop, carrying seven mutations, and showing a melting temperature 15°C higher than that of wild-type (Tm ~105°C), and 400-fold higher kcat/Km for syringol when compared to 2B3 variant. A comprehensive biochemical analysis of hit variants from the in vitro evolution enabled to differentiate among beneficial, neutral and deleterious mutations, showing the path to construct a variant, PheLac, carrying only four mutations (three of them near the Cu centers) and with a higher enzymatic activity. We have investigated the catalytic potential of this variant using a set of representative lignin-related phenolic compounds, including syringyl, guaiacyl, and hydroxybenzene derivatives at pH 8. The results show that this evolved variant has similar or higher turnover numbers than the model CotA-laccase, with the advantage of being significantly more thermostable. The scale-up reaction using the best substrate for McoA hit, sinapyl alcohol, was performed, and syringaresinol, a product with medicinal value and as a building block of biopolymers, was identified using NMR. So far, no studies report this product's synthesis using bacterial laccases. Enzymatic valorization of lignin monomers from phenolic platform chemicals is envisaged as one of the potential environmentally friendly breakthrough applications to valorize lignin bio-wastes.

Acknowledgments: This work was supported by the Fundação para a Ciência e Tecnologia, Portugal, by project grants (PTDC/BII-BBF/29564/2017, EXPL/BIA-BQM/0473/2021 and 2022.02027.PTDC). B-Ligzymes (GA 824017) from the European Union's Horizon 2020 Research and Innovation Program is also acknowledged for funding a secondment of P. Borges to Zymvol, Barcelona, SP.

## FIGURES

### FIGURE 1

### FIGURE 2


## KEYWORDS

Hyperthermophiles | Directed evolution | Enzyme catalysis | Lignin Valorization


## BIBLIOGRAPHY


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