BIOTRANS
LA ROCHELLE 2023

N°1400 / PC
TOPIC(s) : Enzyme discovery and engineering

DIRECTED EVOLUTION OF NOV1 DIOXYGENASE TO ENHANCE THE PRODUCTION OF LIGNIN-DERIVED VANILLIN

AUTHORS
Mario DE SIMONE / ITQB-UNIVERSIDADE NOVA DE LISBOA, AV. DA REPÚBLICA, OEIRAS
Lígia O. MARTINS / ITQB-UNIVERSIDADE NOVA DE LISBOA, ITQB NOVA AV. DA REPÚBLICA, OEIRAS

PURPOSE OF THE ABSTRACT
NOV1 from Novosphingobium aromaticivorans is a non-heme iron-dependent dioxygenase that has activity on lignin-derived phenolics, such as isoeugenol. NOV1 oxidatively cleaves the C=C bond of the propenyl functional group of isoeugenol and produces vanillin without adding external coenzymes. The enzymatic conversion of lignin-derived isoeugenol to vanillin, catalyzed by NOV1, can potentially replace the current petroleum-based synthesis and simultaneously valorize lignin biowaste.

In this work, we applied directed evolution (DE) to improve NOV1 activity towards isoeugenol and its thermal stability, tailoring a more suitable enzyme for industrial applications. Libraries of variants were constructed by error-prone PCR and high throughput screening assays in 96-well plates were developed and validate to test their activity and stability. The best hits from each generation were purified to characterize their kinetic parameters and assess kinetic stability. After three rounds of DE, the best hit 16G3, harbouring 4 non-synonymous mutations, shows a 4-fold increased turnover rate (kcat) and 2.5-fold higher catalytic efficiency (kcat/Km), as compared to wild-type. During the evolution, the low kinetic stability of NOV1 wild-type, which previous studies determined to be linked to the iron cofactor loss [1], was also improved. 16G3 variant displays about 40-fold longer half-life time at 25°C, compared to the wild-type. Overall, these preliminary results show the potential of applying DE to engineer the NOV1 enzyme for tailored properties and to elucidate the structure-function relationship underlying the effect of each mutation.

Acknowledgments: This project has received funding from the Bio-based Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation program under grant agreement No 837890. MDS acknowledges a Ph.D. grant (2020.08246.BD) from Fundação para a Ciência e Tecnologia.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Protein engineering | Biocatalysis

BIBLIOGRAPHY

[1] De Simone M, Alvigini L, Alonso-Cotchico L, Brissos V, Caroli J., Lucas MF, Monza E, Pinho Melo E, Mattevi A, and Martins LO. Rationally Guided Improvement of NOV1 Dioxygenase for the Conversion of Lignin-Derived Isoeugenol to Vanillin. Biochemistry 2023 62 (2), 419-428 DOI: 10.1021/acs.biochem.2c00168.