BIOTRANS
LA ROCHELLE 2023

N°1368 / PC
TOPIC(s) : Enzyme discovery and engineering / Industrial biocatalysis

EXPLORING THE EVOLUTIONARY LANDSCAPE OF PSGALOX, A BACTERIAL GALACTOSE OXIDASE

AUTHORS

Tiago LOPES / ITQB NOVA, AVENIDA DA REPÚBLICA, OEIRAS
André TABORDA / ITQB NOVA, AVENIDA DA REPÚBLICA, OEIRAS
Carolina DIAS / ITQB NOVA, AVENIDA DA REPÚBLICA, OEIRAS
João COSTA / ITQB NOVA, AVENIDA DA REPÚBLICA, OEIRAS
Corresponding author : Lígia MARTINS / lmartins@itqb.unl.pt

PURPOSE OF THE ABSTRACT

Galactose oxidases (GalOxs) are highly attractive enzymes because they efficiently combine oxygen with high specificity and regioselectivity toward carbohydrate substrates.[1] These monomeric metalloenzymes have a copper ion as a cofactor and are secreted by various filamentous fungi, with GalOx from Fusarium graminearum being the best-studied representative. These enzymes' potential include biotechnological applications in small molecule synthesis, oxygen removal, biosensors, and cell surface glycoprotein modification.[2] While galactose oxidases from fungal origins have been vastly studied, there is a growing interest in GalOxs from bacterial origin due to its ease of production and engineering. PsGalOx, an enzyme from Pseudoarthrobacter siccitolerans, is the only known bacterial galactose oxidase.

This work unravels the properties of PsGalOx while tailoring its properties through enzyme engineering approaches. The research seeks to improve the understanding of PsGalOx's catalytic and stability mechanisms by examining the structure-to-function and characterizing the wild-type and variants. To accomplish this, directed evolution was applied recurring to random mutagenesis through error-prone PCR (epPCR). The workflow was developed recurring to two high-throughput screening methodologies, 'activity-on-plate' and 96-well plate liquid screening, which were successfully optimized and validated. Additionally, it was also explored the improvement of PsGalOx‘s solubility and production yields. Multiple rounds of engineering led to the identification of the variant 11C7 which shows close to 20-fold higher protein production yields as compared with the wild-type and 10-fold increased catalytic efficiency (kcat/Km) for D-galactose. We are currently continuing with the evolution of this enzyme to further enhance its catalytic activity and exploring further the effects of directed evolution on its solubility. These findings will contribute to a better understanding of GalOx from different organisms, revealing novel or improved properties to be explored in various fields

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Copper Radical Oxidases | Enzyme engineering | Directed evolution | Galactose oxidase

BIBLIOGRAPHY

1. Savino, S. and Fraaije, M. W. (2021) The vast repertoire of carbohydrate oxidases: An overview. Biotechnol Adv. 51
2. Pedersen, A. T., Birmingham, W. R., Rehn, G., Charnock, S. J., Turner, N. J. and Woodley, J. M. (2015) Process Requirements of Galactose Oxidase Catalyzed Oxidation of Alcohols. Org Process Res Dev. 19, 1580-1589