BIOTRANS
LA ROCHELLE 2023

N°1501 / PC
TOPIC(s) : Enzyme discovery and engineering

Biocatalytic Characterization of an Alcohol Dehydrogenase Variant Deduced from Lactobacillus kefir in Asymmetric Hydrogen Transfer

AUTHORS

Aleksandra RUDZKA / WARSAW UNIVERSITY OF TECHNOLOGY, NOAKOWSKIEGO 3, WARSAW
Beata ZDUN / WARSAW UNIVERSITY OF TECHNOLOGY, NOAKOWSKIEGO 3, WARSAW
Natalia ANTOS / WARSAW UNIVERSITY OF TECHNOLOGY, NOAKOWSKIEGO 3, WARSAW
Lía MARTÍNEZ MONTERO / UNIVERSITY OF GRAZ, HEINRICHSTRASSE 28, GRAZ
Tamara REITER / UNIVERSITY OF GRAZ, HEINRICHSTRASSE 28, GRAZ
Wolfgang KROUTIL / UNIVERSITY OF GRAZ, HEINRICHSTRASSE 28, GRAZ
Paweł BOROWIECKI / WARSAW UNIVERSITY OF TECHNOLOGY, NOAKOWSKIEGO 3, WARSAW

PURPOSE OF THE ABSTRACT

Optically active alcohols are key chiral building blocks supporting the synthesis of high-value-added products. Therefore, it is essential to develop practical, selective, scalable and environmentally friendly methods to obtain them. Unfortunately, most methods for the synthesis of chiral alcohols have significant limitations related mainly to low reaction yields and the high cost of product isolation and purification. Therefore, desymmetrization of prochiral or meso-substrates is considered as one of the most valuable transformations, while the vast majority of efficient and selective biocatalysts capable of reducing carbonyl compounds are alcohol dehydrogenases (E. C. 1.1.1) (ADHs). Hence, hydrogen-transfer biocatalysts for preparing optically pure chiral secondary alcohols are of great interest, especially for sterically demanding ketones.

This work reports on the biocatalytic potential of an anti-Prelog (R)-specific ADH variant from Lactobacillus kefir (Lk-ADH-E145F-F147L-Y190C named Lk-ADH Prince) [1] used as an E. coli/ADH whole-cell biocatalyst and its characterization for the stereoselective reduction of prochiral carbonyl substrates. During our studies, crucial parameters of the enzymatic reaction (i.e., reaction medium, cofactor dependence assessment, tolerance to organic co-solvent, and substrate loading) were optimized using pentoxyphylline active agent as a model prochiral ketone. Next, we investigated the substrate scope for Lk-ADH Prince by employing 34 carbonyl derivatives in hydrogen transfer reactions. Our results revealed that E. coli/Lk-ADH Prince is highly stereoselective biocatalyst capable of reducing structurally diverse ketones, providing optically active alcohols with excellent enantiomeric excesses (up to >99%), in high yields (up to 91%) and at relatively high substrate concentration (up to 100 mM) without requiring the addition of costly NADPH cofactor.

Acknowledgements: This research was funded by the Warsaw University of Technology within the ‘Excellence Initiative: Research University (IDUB)' programme in the framework of the ‘Young PW' starting grant. A.R. and B.Z. are grateful to the IDUB project ('Scholarship Plus' programme) for providing a research fellowship. The University of Graz and the Field of Excellence BioHealth are acknowledged for financial support. Joerg H. Schrittwieser from the Institute of Chemistry, University of Graz, is thanked for his suggestion to select this enzyme from literature and valuable discussion.

FIGURES

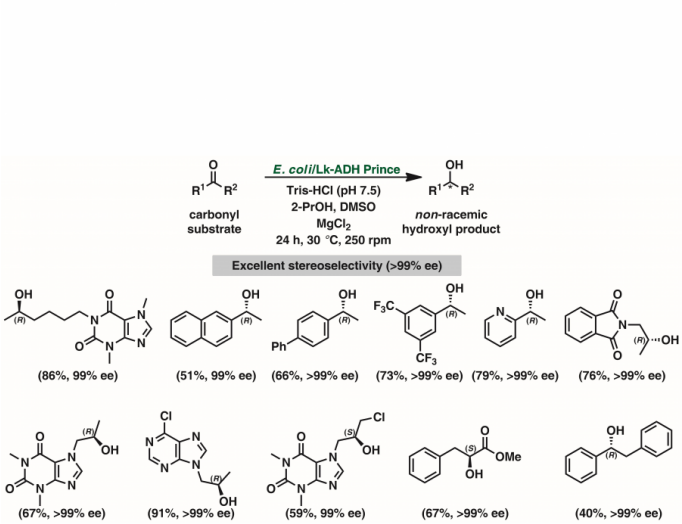

FIGURE 1

Asymmetric reduction of prochiral ketones using E. coli/Lk-ADH Prince.

FIGURE 2

KEYWORDS

Chiral alcohols | Alcohol dehydrogenases | Lactobacillus kefir | Engineered enzyme variant

BIBLIOGRAPHY

[1] Emmanuel, M. A., Greenberg, N. R., Oblinsky, D. G., Hyster, T. K., Nature, 2016, 540, 414-417.