BIOTRANS
LA ROCHELLE 2023

N°1290 / PC
TOPIC(s) : Enzyme discovery and engineering

Discovery of Bacterial Reductive Aminases via Enzyme Mining

AUTHORS

Ewald JONGKIND / DELFT UNIVERSITY OF TECHNOLOGY, VAN DER MAASWEG 9, DELFT
Caroline PAUL / DELFT UNIVERSITY OF TECHNOLOGY, VAN DER MAASWEG 9, DELFT
Melis TEKE / DELFT UNIVERSITY OF TECHNOLOGY, VAN DER MAASWEG 9, DELFT
Marcel VAN DEN BROEK / DELFT UNIVERSITY OF TECHNOLOGY, VAN DER MAASWEG 9, DELFT

PURPOSE OF THE ABSTRACT

The synthesis of chiral amines is highly valuable for the production of pharmaceutical drugs. Over the last decade, several enzyme classes have been employed to produce mainly chiral primary amines with high efficiency and enantioselectivity. Since 2017, Turner and co-workers have characterized several fungal reductive aminases (RedAms) that catalyze both imine formation and asymmetric reduction, resulting in secondary and tertiary amines.[1] Several bacterial imine reductases (IREDs) also displayed reductive aminase activity, albeit with different catalytic residues in their active site than those of RedAms.[2] Only one recent example of a bacterial RedAm sequence has been reported so far.[3] We envisioned to explore new RedAm sequences from bacterial organisms in genomic databases. After an initial BLAST search, we discarded all hits with a sequence identity of fifty percent or higher to exclude fungal RedAms. Using EnzymeMiner,[4] we selected seven hits from different organisms that contained the reported catalytic residues in their active site. We produced the new enzymes by recombinant expression in E. coli, and observed reductive amination activity towards different carbonyl substrate and amine donor combinations. We also applied an Hidden Markov-Model to search for hits in our in-house Rhodococcus database. We retrieved a sequence that gave a specific activity of 6 U/mg towards hexanal and allylamine, and in contrast to other RedAms, showed higher activity at pH 7.0 than at 9.0. Further screening of different carbonyl substrates and amine donors is ongoing.

FIGURES

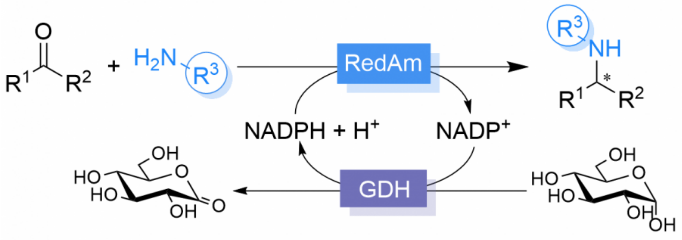

FIGURE 1

Reductive amination of ketones and aldehydes catalysed by potential bacterial reductive aminases, using a glucose dehydrogenase for cofactor recycling.

.

FIGURE 2

KEYWORDS

Reductive amination | Biocatalysis

BIBLIOGRAPHY

[1] G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan, N. J. Turner, Nat. Catal. 2017, 9, 961-969.

[2] S. L. Montgomery, A. Pushpanath, R. S. Heath, J. R. Marshall, U. Klemstein, J. L. Galman, D. Woodlock, S. Bisagni, C. J. Taylor, J. Mangas-Sanchez, J. I. Ramsden, B. Dominguez, N. J. Turner, Sci. Adv. 2020, 6, eaay9320.

[3] K. Zhang, Y. He, J. Zhu, Q. Zhang, L. Tang, L. Cui, Y. Feng, Front Bioeng Biotechnol 2021, 9, 798147.

[4] J. Hon, S. Borko, J. Stourac, Z. Prokop, J. Zendulka, D. Bednar, T. Martinek, J. Damborsky, Nucleic Acids Res. 2020, 48, W104-W109.