

SYNTHESIS OF β -HYDROXY- α -AMINOACIDS UTILIZING L-THREONINE (TRANS)ALDOLASES

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β -Hydroxy- α -aminoacids (β -HAAs) are a class of compounds widely used as intermediates in pharmaceutical synthesis (antibiotics, antiviral drugs), with the capacity to be incorporated into therapeutic peptides or functional proteins [1]. β -HAAs are typically obtained via chemical synthesis, although this process is unfavourable due to the use of several blocking groups during synthesis [2]. An alternative approach involves an enzymatic synthesis using L-threonine aldolases and transaldolases, which catalyse an aldol reaction between glycine or L-threonine and an aldehyde [3].

In this study, substrate selectivity was investigated using L-threonine aldolases from *Neptunomas marina* and *Cellulosilyticum* sp. (NmLTA and CsLTA) and L-threonine transaldolase from *Pseudomonas fluorescens* (ObiH). The substrate scope was evaluated using whole-cell biocatalysis, discovering that among the 23 aldehydes tested, 9 were suitable substrates for at least one enzyme. The analysis of enzymatic reactions showed that NmLTA and CsLTA catalyse the aldol reaction with pyridine-carbaldehydes (13-22%) as well as large aromatic aldehydes (3-7%), while ObiH was also active towards formyl-indoles (44-84%).

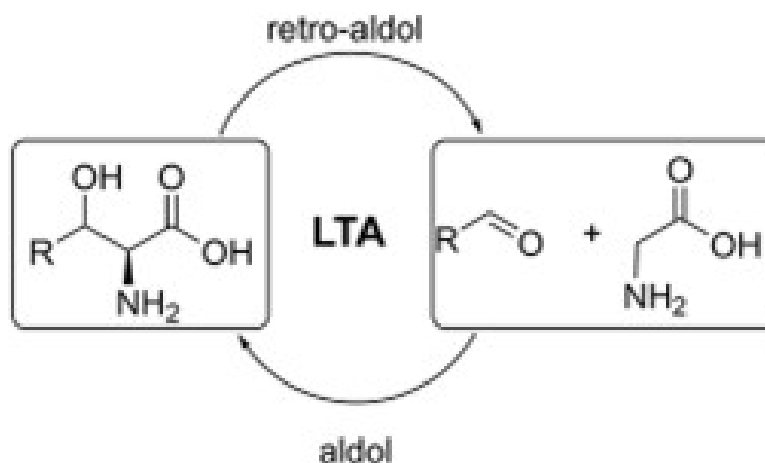


Fig. 1. Fig. 1 L-Threonine aldolase catalysed reactions [4].

[1] Chen, Q., Wang, J., Zhang, S., Chen, X., Hao, J., Wu, Q., & Zhu, D. "Discovery and directed evolution of C-C bond formation enzymes for the biosynthesis of β -hydroxy- α -amino acids and derivatives". *Critical Reviews in Biotechnology*, Vol. 44, Issue 8, pp. 1495-1514. 2024.

[2] He, Y., Li, S., Wang, J., Yang, X., Zhu, J., Zhang, Q., Cui, L., Tan, Z., Yan, W., Zhang, Y., Tang, L., Da, L. T., & Feng, Y. "Discovery and Engineering of the L-Threonine Aldolase from *Neptunomonas marina* for the Efficient Synthesis of β -Hydroxy- α -amino Acids via C-C Formation". *ACS Catalysis*, Vol. 13, Issue 11, pp. 7210-7220. 2023.

[3] Wang, S., & Deng, H. "Peculiarities of promiscuous L-threonine transaldolases for enantioselective synthesis of β -hydroxy- α -amino acids". *Applied microbiology and biotechnology*, vol. 105, no. 1, pp 1-11. 2020.

[4] Fesko, K. "Comparison of L-threonine aldolase variants in the aldol and retro-aldol reactions". *Frontiers in Bioengineering and Biotechnology*, vol. 7, pp 1-12. 2019.