BIOTRANS
LA ROCHELLE 2023

N°477 / PC
TOPIC(s) : Enzyme discovery and engineering / Synthetic biology, metabolic engineering

The Discovery and Characterization of a Fungal Aldolase with High Formaldehyde Affinity

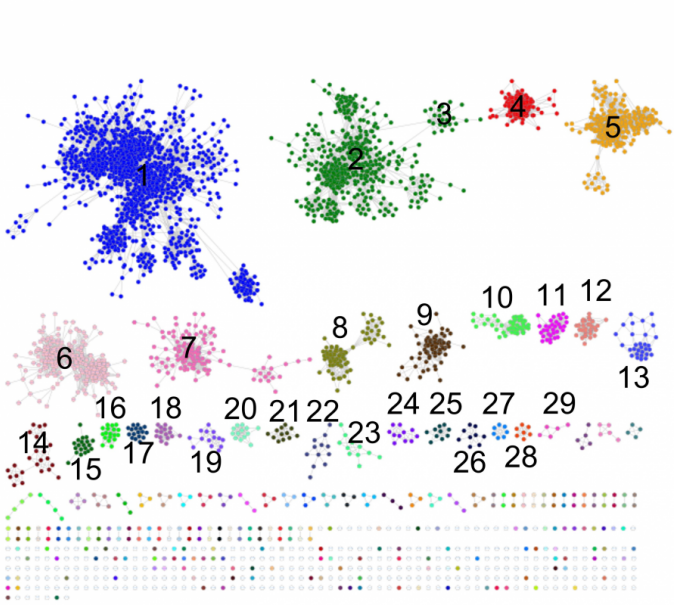
AUTHORS

Yonghui XIE / TIANJIN INSTITUTE OF INDUSTRIAL BIOTECHNOLOGY,CHINESE ACADEMY OF SCIENCES, 32 XI QI DAO, TIANJIN AIRPORT ECONOMIC AREA, TIANJIN
Hongjun DONG / TIANJIN INSTITUTE OF INDUSTRIAL BIOTECHNOLOGY,CHINESE ACADEMY OF SCIENCES, 32 XI QI DAO, TIANJIN AIRPORT ECONOMIC AREA, TIANJIN
Xiang SHENG / TIANJIN INSTITUTE OF INDUSTRIAL BIOTECHNOLOGY,CHINESE ACADEMY OF SCIENCES, 32 XI QI DAO, TIANJIN AIRPORT ECONOMIC AREA, TIANJIN
Corresponding author : Xiang SHENG / shengx@tib.cas.cn

PURPOSE OF THE ABSTRACT

The homoserine cycle is an artificially designed methanol assimilation pathway, relying on two promiscuous formaldehyde-condensing aldolase reactions [1]. It is expected to outperform the generally used natural pathways such as the RuMP cycle and the serine cycle. However, previous study revealed that the low activity of pyruvate aldolase, more specifically the binding affinity of formaldehyde, represents a limiting factor for the efficiency of the homoserine cycle [1]. In the present study, we used a genomic enzymology web tool [2], called Enzyme Function Initiative-Enzyme Similarity Tool (EFI-EST), to mine new aldolases for the reversible aldol condensation of pyruvate with formaldehyde among the Class II pyruvate aldolase. On the basis of the generated sequence similarity networks, we segregated the family into different functional clusters and selected one from each cluster for the characterization of formaldehyde-condensing activity by the colorimetric method developed by ourselves. A number of active aldolases were identified. The one with the highest affinity of formaldehyde was from fungi and the measured Km value was 0.34mM, which is two orders of magnitude lower than those precedingly reported [3,4]. The multiple sequence and structure alignment of aldolases indicate that a hydrophobic flexible loop of the adjacent subunit may play an important role in the improved binding affinity of pyruvate aldolase toward formaldehyde. We thus identified a novel aldolase, whose condensation rate and binding affinity of formaldehyde are high enough to be physiologically applicable, which opens the way for the application of this unnatural condensation reaction in vivo.

FIGURES



name	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M ($M^{-1}s^{-1}$)
2-Ald3	46 ± 7	28 ± 6	(1.7 ± 0.4) × 10^3
2-Ald4	6.1 ± 2.4	110 ± 50	(5.5 ± 3.3) × 10^1
2-Ald5	1.3 ± 0.1	0.34 ± 0.06	(3.7 ± 0.7) × 10^3
2-Ald9	1.3 ± 0.1	6.5 ± 0.7	(2.0 ± 0.2) × 10^2

FIGURE 1

Sequence Similarity Networks (SSNs) for sequences from the Class II pyruvate aldolase generated with EFI-EST.

Alignment score is 60, 45% sequence identity. we selected one from each cluster for the characterization of formaldehyde-condensing activity.

FIGURE 2

The Kinetic Properties of Aldolases for the condensation of pyruvate with formaldehyde.

Aldolase activity was detected by the colorimetric method developed by ourselves, relying on monitoring the consumption of formaldehyde.

KEYWORDS

Formaldehyde assimilation | Class II pyruvate aldolase | Homoserine cycle | Enzyme discovery

BIBLIOGRAPHY

[1] He, H.; Hoper, R.; Dodenhoft, M.; Marliere, P.; Bar-Even, A., Metab. Eng. 2020, 60, 1-13.

[2] Gerlt, J. A., Biochemistry. 2017, 56, 4293-4308.

[3] Bosch, S.; Sanchez-Freire, E.; del Pozo, M. L.; Cesnik, M.; Quesada, J.; Mate, D. M.; Hernandez, K.; Qi, Y.; Clapes, P.; Vasic-Racki, D.; Findrik Blazevic, Z.; Berenguer, J.; Hidalgo, A., ACS Sustain. Chem. Eng. 2021, 9, 5430-5436.

[4] Hernandez, K.; Bujons, J.; Joglar, J.; Charnock, S. J.; Dominguez de Maria, P.; Fessner, W. D.; Clapes, P., ACS Catalysis. 2017, 7, 1707-1711.