ENGINEERING OF HALOHYDRIN HYDROGEN-HALIDE-LYASE (H-LYASE) FOR EFFICIENT L-CARNITINE PRODUCTION

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L-carnitine is a beta-hydroxy carboxylic acid known as a dietary supplement with the effect of fat-burning or anti-fatigue. Although several production processes of L-carnitine are proposed including fermentation, chiral resolution or asymmetric synthesis, there are disadvantages with each from the point of view of yield, side-reactions or productivity. We have engaged in technology development of efficient enzymatic production of various chemicals for years, represented with the world's first industrialized bioprocess for acrylamide. Leveraging our specialty including proprietary nitrile-related enzymes and associated technologies, we attempted to develop a further efficient production process of L-carnitine (Figure 1).

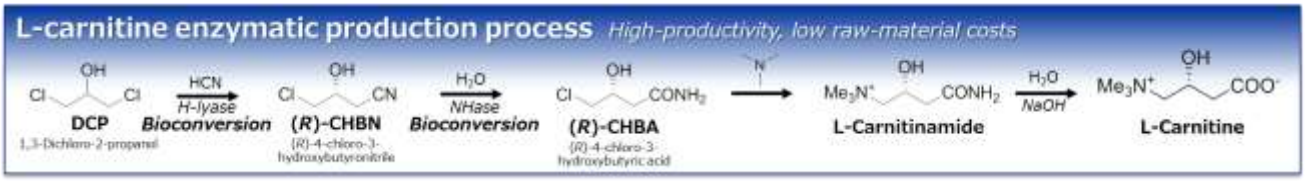

Figure 1 – L-carnitine enzymatic production process

Halohydrin hydrogen-halide-lyase (H-lyase) is the enzyme capable of catalyzing the reversible dehalogenation of vicinal haloalcohols with formation of the corresponding epoxides (Figure 2). We envisioned the enzymatic process for L-carnitine production using HheB, a B-type H-lyase from *Corynebacterium* sp. N-1074, which can convert 1,3-dichloro-2-propanol (DCP) and hydrogen cyanide into 4-chloro-3-hydroxybutyronitrile (CHBN) with *R*-preference [1]. Although its unique feature and enantioselectivity were promising as the biocatalyst for our L-carnitine process, its performance was not adequate from the point of view of industrial use. Thus, we attempted to improve the tolerance for product-inhibition and enantioselectivity of H-lyase by enzyme engineering.

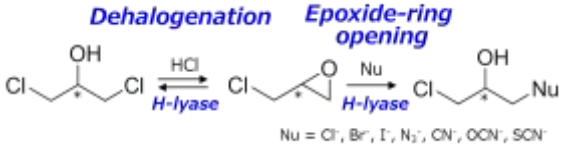

Figure 2 – Reaction catalyzed by H-lyase

Regarding alleviation of product-inhibition, we adopted a random approach. After screening over 20,000 variants by evaluating activities in the presence of the product, that is (*R*)-CHBN, a few variants with alleviated inhibition were successfully obtained. Especially, D199H showed alleviated product inhibition not only by (*R*)-CHBN but also by chloride ion, resulting in increased accumulation of (*R*)-CHBN. As for enantioselectivity improvement, in contrast, a structure-based approach was conducted. The crystal structure of HheB led us to identify some key residues possibly affecting the enantioselectivity. Through saturation mutagenesis on focused residues, several variants with improved enantioselectivity were discovered. Among them, F71-variants showed remarkable improvement of enantioselectivity with increased accumulation of product, in spite of reduced initial activity. Eventually, by combining those useful mutations, we succeeded in evolving wild-type HheB into the practical biocatalyst for L-carnitine production with industrial-level performance [2, 3].

References

[1] *Tetrahedron*, 50 (41), 11821-11826 (1994).
[2] Patent WO/2008/108466
[3] *J. Biosci. Bioeng*., 122 (3), 270-275 (2016)