Enzyme Engineering of Alcohol Dehydrogenase from *Geotrichum candidum* NBRC 4597 (*Gc*APRD) with enhanced activity and enantioselectivity

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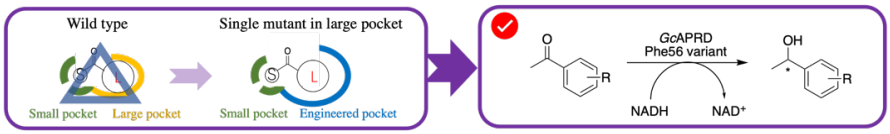
The alcohol dehydrogenases (ADHs)-catalyzed asymmetric reduction of ketones to produce enantiopure alcohols is beneficial for pharmaceutical applications. An ADH from *Geotrichum candidum* NBRC 4597 (*G. candidum* acetophenone reductase, *Gc*APRD) has been reported as a novel enzyme that can reduce prochiral ketones following Prelog's rule to their corresponding (*S*)-alcohols.[1,2] The previous study demonstrated that the substrate specificity and enantioselectivity of *Gc*APRD could be enhanced and changed by mutating a bulky residue, Trp288, in the small binding pocket to a smaller one *via* site-directed mutagenesis.[3] Two mutants, Trp288Ala and Trp288Val, showed very promising results.

Despite this research, the *Gc*APRD-catalyzed reduction of the "bulky-bulky" ketones, owning both bulky substituents to the carbonyl carbon, is still limited. To overcome this limitation, double mutation in both small and large binding pocket is necessary. To construct the best double mutants, it is necessary to find the best single mutant in the large binding pocket. Site-directed mutagenesis was performed at Phe56, which locates at the entrance of the large binding pocket. It is noteworthy that Phe56 mutants, such as Phe56Val and Phe56Ile, can maintain the activity on acetophenone reduction and show the increased relative activity on halogenated acetophenone derivatives reduction compared with the wild type.



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