Improvement of Activity of Alcohol Dehydrogenase from *Geotrichum candidum* for Reduction of Bulky Ketones *via* Enzyme Engineering

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Alcohol dehydrogenase from *Geotrichum candidum* NBRC 4597 (*G. candidum* acetophenone, *Gc*APRD) has been reported as a novel enzyme that can reduce prochiral ketones following Prelog's rule to their corresponding (*S*)-alcohols.[1] Although *Gc*APRD wild type and Trp288 mutants showed activity and enantioselectivity on various ketones,[2] catalyzing the reduction of bulky-bulky ketones remains challenging. To solve this problem, further mutagenesis is necessary. Based on the previous docking simulation study, a double mutant *Gc*APRD with site-directed mutagenesis at Trp288 and Phe56 was shown the potential. Before examining a double mutant, besides Trp288, various Phe56 mutants were also examined.

This research constructed three *Gc*APRD mutants (Phe56Val, Phe56Ile, Phe56His) to find a Phe56 mutant with high activity and stereoselectivity. Using acetophenone and its halogenated analogs as substrates, Phe56Val and Phe56Ile showed enhanced absolute specific activity toward some of the substrates tested. Moreover, Phe56Ile also showed strict (*S*)-stereoselectivity with high *ee* (>99%) in asymmetric reductions, and its mechanism was examined by docking simulation. These results indicated that making site-directed mutagenesis on Phe56 still maintains strict (*S*)-stereoselectivity of the enzyme.

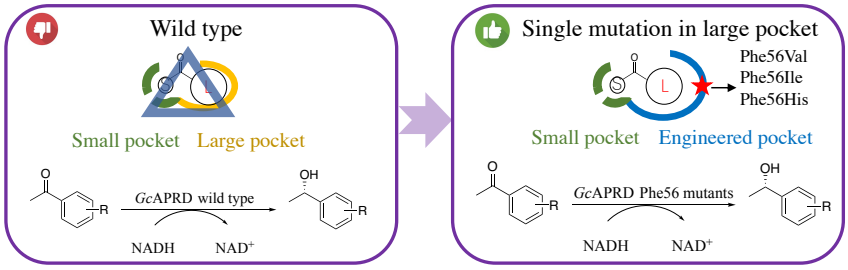

Fig. 1 Reduction of acetophenone and its halogenated analogs by *G. candidum* acetophenone reductase wild type and mutants.

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2) Koesoema A A, Standley D M, Ohshima S, *et al*. *Tetrahedron Letters*, **2020**, *61*, 151820.