APPLICATION OF DIRECTED DIVERGENT EVOLUTION STRATEGY IN NATURAL PRODUCT BIOSYNTHESIS

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Genetic diversity is a result of evolution, enabling multiple ways for one particular physiological activity. Here, we introduce this strategy into bioengineering. We design two hydroxytyrosol biosynthetic pathways using tyrosine as substrate. We show that the synthetic capacity is significantly improved when two pathways work simultaneously comparing to each individual pathway. Next, we engineer flavin-dependent monooxygenase HpaBC for tyrosol hydroxylase, tyramine hydroxylase, and promiscuous hydroxylase active on both tyrosol and tyramine using directed divergent evolution strategy. Then, the mutant HpaBCs are employed to catalyze two missing steps in the hydroxytyrosol biosynthetic pathways designed above. Our results demonstrate that the promiscuous tyrosol/tyramine hydroxylase can minimize the cell metabolic burden induced by protein overexpression and allow the biosynthetic carbon flow to be divided between two pathways. Thus, the efficiency of the hydroxytyrosol biosynthesis is significantly improved by rearranging the metabolic flux among multiple pathways.