Engineering prenyltransferase NphB for enhancing regioselectivity and catalytic activity toward Olivetolic Acid

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Cannabinoids are a class of natural products originally isolated from the plant Cannabis sativa. The legalization trend of the compounds from C. sativa has made a global surge in demand for cannabinoid-derived medications. Nevertheless, their utilization is hindered by the lack of cost-effective cannabinoid production methods. To address the issue, research employing a synthetic biology approach to produce various cannabinoids enzymatically is currently underway. Cannabigerolic acid (CBGA) is a pivotal precursor which can be converted into valuable cannabinoids such as Tetrahydrocannabinolic acid (THCA), Cannabidiolic acid (CBDA), and Cannabichromenic acid (CBCA). NphB is a soluble aromatic prenyltransferase from Streptomyces sp. which has been shown to be able to prenylate diverse aromatic substrates including olivetolic acid (OA) to form CBGA. However, during CBGA production by NphB, unwanted side product has been also produced, consequently reducing yield of CBGA. In this presentation, we engineered NphB to enhance regioselectivity and catalytic efficiency toward OA via computational rational design. Based on the structural analysis and information from previously reported mutants, nine residues have been selected for analysis using the online available tool named FuncLib. Through the analysis, critical residues influencing regioselectivity and activity were identified. The variants including the residues produced CBGA over 15-fold increase than wild-type without side product. Our NphB variants hold promise for producing valuable cannabinoids with improved regioselectivity and catalytic activity, thereby advancing cannabinoid-based therapeutics.