

Title: Cell-free enzymatic biosynthesis of cannabinoids

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Project Goals: To design cell-free enzymatic systems to produce complex natural products, like cannabinoids.

Abstract

Prenylation of natural compounds adds structural diversity, alters biological activity, and enhances therapeutic potential. Because prenylated compounds often have a low natural abundance, alternative production methods are needed. Metabolic engineering enables natural product biosynthesis from inexpensive biomass, but is limited by complexity of secondary metabolite pathways, intermediate and product toxicities, and substrate accessibility. Alternatively, enzyme catalyzed prenyl transfer provides excellent regio- and stereo-specificity, but requires expensive isoprenyl pyrophosphate substrates. Here we develop a flexible cell-free enzymatic prenylating system that generates isoprenyl pyrophosphate substrates from glucose to prenylate an array of natural products. The system provides an efficient route to cannabinoid precursors cannabigerolic acid (CBGA) and cannabigerovarinic acid (CBGVA) at > 1 g/L, and a single enzymatic step converts the precursors into cannabidiolic acid (CBDA) and cannabidivarinic acid (CBDVA) respectively. Cell-free methods may provide a powerful alternative to metabolic engineering for chemicals that are hard to produce in living organisms.

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