

Regio-specific hydroxylation of isoflavonoids using bacterial cytochrome P450s

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The whole cell bio-conversion of daidzein was examined for ortho-hydroxylation using bacterial cytochrome P450s. The cytochrome P450s belong to a family of ubiquitous heme-containing monooxygenases and catalyze diverse reactions, such as hydroxylation, epoxidation, reduction, oxidation, O-demethylation, desulfonation and peroxidation reactions. Among the various reactions of cytochrome P450, hydroxylation of isoflavones with high regio- and stereo-selectivity is of special interest for biotechnological applications. To identify monooxygenases which play a key role for the ortho-hydroxylation, P450 pools were constructed in *E. coli* BL21 (DE3) host system and each P450 was co-expressed with redox proteins, camA and camB from *Pseudomonas putida*, to produce ortho-dihydroxyisoflavones (ODIs). The major hydroxylated products were 3'-ODI, 6-ODI and 8-ODI which were mono-hydroxylated at ortho position of hydroxyl group of daidzein. And quantitative analysis of reaction profiles revealed that 3'-hydroxylation of daidzein B-ring was catalyzed by CYP105D1 of *Streptomyces avermitilis* MA-4680 and hydroxylation of each 6- and 8-position of daidzein A-ring were catalyzed by each nfa12130 and nfa33880 P450 genes of *Nocardia farcinica* IFM10152, respectively.