GENERATION OF HUMAN DRUG METABOLITES BY BACTERIAL CYTOCHROME P450 BM3 MUTANTS
Chul-Ho Yun
School of Biological Sciences and Technology, Chonnam National University, Gwangju 500-757, REPUBLIC OF KOREA (E-mail: chyun@jnu.ac.kr)

We have proposed that cytochrome P450 BM3 from Bacillus megaterium can be developed as a biocatalyst with human P450 activities oxidizing clinical drugs (1). In recent studies, several P450 BM3 mutants were found to generate human drug metabolites by oxidizing various drugs including trimethoprim, clozapine, diclofenac, acetaminophen, 3,4-methylenedioxyxymethamphetamine, dextromethorphan, resveratrol, verapamil, astemizole, phenacetin, coumarin, terpenes, geranylacetone, nerylacetone, (4R)-limonene, and (+)-valencene (2-5). These P450 BM3 mutants were generated by directed evolution and rational design and found to have much higher activity and stability than human P450 enzymes and P450 BM3 wild-type. Scalable and cost-effective production of human drug metabolites is required for the studies of drug toxicity and efficacy during the process of drug discovery and development (6). FDA guidelines also indicate that human and animal metabolites are required during toxicology test and safety assessment (http://www.questpharm.com/FDA.html). We found that human metabolite piceatannol can be generated from the anti-cancer preventive agent resveratrol by P450 BM3 (2).

However, piceatannol cannot be made from its substrate, resveratrol, by human P450 enzymes as piceatannol is a potent inhibitor for the human P450 catalyzed reactions. High active mutants of P450 BM3 for human P450 substrates were selected from mutant library using indigo formation (4). The P450 BM3 mutants usually did not show selectivity towards each human drug substrate although they have much higher oxidation activity than those of human P450 enzymes. If any P450 BM3 mutants have high activity to certain substrate, they usually have high monooxygenase activities to most of other substrates. At present it seems to be difficult to make specialized P450 BM3 mutants for a certain human P450 reaction. I suggest that P450 BM3 mutants can be used to produce human drug metabolites, which are essential compounds for drug discovery and development.

References:

Biography
2004-present, Professor, School of Biological Sciences and Technology, Chonnam National University, Gwangju, Korea; 1992-2004, Professor, Division of Biological Sciences, Pai-Chai University, Daejeon, Korea; 1990-1992, Research Associate, Department of Biochemistry, Vanderbilt University, Nashville, TN, USA; 1990, Ph.D. and 1987, M.S., Department of Biological Sciences and Engineering, KAIST, Seoul, Korea; 1985, B.E., Department of Biotechnology, Yonsei University, Seoul, Korea