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Construction of Biocatalyst Library for Combinatorial Biocatalysis

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The evolution of life on Earth has occurred through enzyme-catalyzed, combinatorial organic synthesis coupled with natural selection of biomolecules with optimal functions. Mimicking this process, combinatorial biocatalysis harnesses the natural diversity of enzymatic reactions for the iterative synthesis of organic libraries, thus enabling the efficient integration of automated synthesis and high-throughput screening for the discovery of new biocactive compounds.

Combinatorial biocatalysis is a powerful tool for generating focused libraries of derivatives, analogs, and possible metabolites from existing lead compounds.

Combinatorial biocatalysis technique, "Biosynthesis and Identification Of Active Compounds Through Iterative Variation" (BIOACTIV), developed at EnzyMed, integrates iterative enzymatic and microbial reactions to create libraries of derivatives from lead compound. BIOACTIV process has proven itself applicable to both small molecules and complex natural products creating diverse variations of their promising leads.

Biocatalysis has demonstrated advantages that stem from the inherent chemo-, regio-, and stereoselectivity of enzymes. Thus combinatorial biocatalysis technology provides the biocatalytic platform to access compounds difficult or impractical to achieve by chemical methods alone. One of the reactions difficult or impractical by chemical methods is the stereospecific and regiospecific hydroxylation.

In this experiment, genomic DNA associated with hydoxylation reaction is manipulated and transferred from a variety of organisms into appropriate host microorganisms (in this research, *Streptomyces lividans*) so that each *Streptomyces lividans* receives a different set of genetic instructions concerning to hydroxylation. The host organisms expressing the added biocatalytic genes were used for the biotransformation of various compounds.

The genes for hydroxylation of phenanthrene (*phdABCD*) from *Nocardioides* sp. KP7, biphenyl (*bphA1A2A3A4*) from *Pseudomonas pseudoalcaligenes* KF707 and *Burkholdelia cepacia*LB400, toluene(*todC1bphA2A3A4*) from *Pseudomonas putida* F1, carotene (*crtZ, crtW*) from *Agrobacteriun aurantiacum,* and zeaxanthin (*Aba2*) from *Nicotiana plumbaginifolia* were manipulated and expressed in *Streptomyces lividans* using streptomyces expression vector pIJ6021. The recombinant *Streptomyces* strains were used for biotransformation of many compounds to generate their hydroxylated products.

These recombimamt streptomyces could be used for the construction of biocatalyst library for the combinatorial biocatalysis. Successive rounds of biocatalysis, or chemoenzymatic steps, increase the diversity in expanding arrays of derivatives. Well suited for modifying natural products and constructing new molecules from simple building blocks, combinatorial biocatalysis efficiently produces novel libraries with an extensive range of biological properties.