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Development of Biocatalysts for Chemical Biotechnology: Case Report and Review of Lipases

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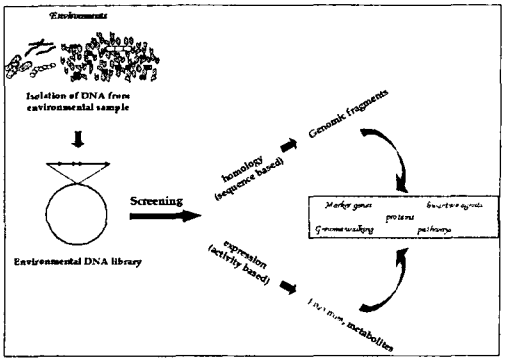
Enzymes are the catalysts evolved in nature to achieve the speed and coordination of a multitude of chemical reaction necessary to develop and maintain life. Since prehistoric times biocatalysis has played a significant role in chemical transformations such as baking, brewing, and cheese production. Today, with over 3000 enzymes identified so far, the huge potential of enzymes is having an increasing impact on biotechnology and organic chemistry. Especially, "chemical biotechnology" is the rapidly growing application of biotechnology to chemical production, and the major field driving chemical biotechnology is "biocatalysis technology" for bulk chemical, pharmaceutical and agrochemical intermediates, including production and isolation of novel enzyme from natural biodiversity, enzyme engineering by directed evolution and rational design, reaction engineering, etc. In fact, biocatalysis and biotransformation could account for 30% of the chemicals business by the year 2050.

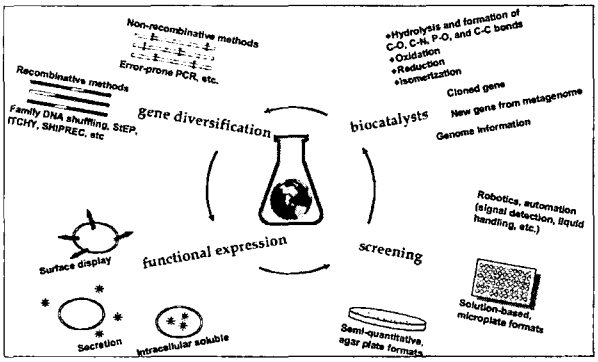
This report, in which an outline of cases of lipases is mainly given, represents the work of chemical biotechnology concerning with the development of biocatalysts. The topics covered are: (1) mechanisms and applications of lipases, (2) lipase production and diversity, (3) screening strategies for lipases, and (4) directed evolution of lipases.

Lipases are the most used enzymes in synthetic organic chemistry, catalyzing the chemo-, region-, and/or stereoselective hydrolysis of carboxylic acid esters or the reverse reaction in organic solvents. Many microbial lipase genes have been cloned from natural producers, including important commercial lipases, and the structural and biochemical characteristics of lipases have been studied. Information on sequences and structures of families of homologous microbial lipases is integrated on the web (Lipase Engineering Database, http://www.led.uni-stuttgart.de/). Development of an efficient and convenient assay system is also essential in the screening of a large number of microorganisms. Lipase screening can be performed, for example, with chromophore released from synthetic substrates such as various *p*-nitrophenyl esters (solution-based, S), synthetic fluorescent substrates (S), triglyceride-emulsified agar plates by detecting the halo formation (agar plate-based, A), pH indicator by detecting the change of pH induced from the hydrolysis of target substrates (A, S).

The classical approach to isolate new enzymes is enrichment and screening of a wide variety of microorganisms, from which the enzymes and the corresponding genes are then recovered. Meanwhile, the alternative way is to employ the isolated environmental DNA and to clone directly functional genes from metagenomic libraries. This approach will be successfully applied for the identification of novel lipase genes.

Recombinant DNA technologies allow for the engineering of lipases for specific applications by altering its enantioselectivity, substrate specificity or general process performance. This can be done either by rational design or by a promising directed protein evolution. The enzymes have evolved over millions of years to be efficient and selective catalysts for the chemical reactions taking place in living systems. Protein engineering can be used to change the properties of these natural catalysts to suit the needs of chemical industry. Much progress has been made by both industrial and academic laboratories, thus allowing us to develop new enzymes for optimal performance by directed evolution.





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