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Review

Strategies for design of improved biocatalysts for industrial applications

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HIGHLIGHTS

- Overview of enzyme bio-prospecting.
- Recent trends in enzyme technologies for biocatalyst improvement.
- Directed evolution and Immobilisation as tools for enzyme improvement.
- Computational strategies for novel biocatalyst design.

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ABSTRACT

Biocatalysts are creating increased interest among researchers due to their unique properties. Several enzymes are efficiently produced by microorganisms. However, the use of natural enzymes as biocatalysts is hindered by low catalytic efficiency and stability during various industrial processes. Many advanced enzyme technologies have been developed to reshape the existing natural enzymes to reduce these limitations and prospecting of novel enzymes. Frequently used enzyme technologies include protein engineering by directed evolution, immobilisation techniques, metagenomics etc. This review summarizes recent and emerging advancements in the area of enzyme technologies for the development of novel biocatalysts and further discusses the future directions in this field.

Contents

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1. Introduction

Enzymes are powerful biocatalysts and enhance the reaction rates of various biological and chemical processes. Recently there is a high requirement for the potent biocatalysts and are considered as eco-friendly alternatives for high value chemical synthesis. Majority of chemical synthesis now uses environmentally risky organic solvents and require high energy for the processes (Mackenzie et al., 2015). As microbial enzymes do not possess any environmentally hazardous nature, and thus provide "cleaner" solutions for the synthesis of bulk chemicals and compounds.

Large number of enzymes has been commercially used in several industries such as leather, textiles, food, paper, food, pharmaceuticals and detergent. Important industrial biocatalysts include lipase (biofuel and pharmaceutical industry), trypsin (leather industry), xylanase (paper and pulp industry), lipoxygenase (food industry), phytase (feed industry), cold active proteases and α-amylase (detergent industry), and hyaluronidase (a pharmaceutical enzyme) (Bhavsar et al., 2013; Plagemann et al., 2013; Yang et al., 2011; Khatri et al., 2016; Rebello et al., 2017; Rai et al., 2017).

The major source of biocatalysts is microorganisms due to the advantage of easy laboratory culturing, natural abundance and rich diversity. Although biocatalysts catalyses a wide number of chemical reactions, they are developed towards the needs of their natural catalysis. Thus, they are not suitable for many of the important catalytic processes and other industrially relevant substrates. Enzymes should possess high activity as well as high specificity and enantio-selectivity towards range of different challenging substrates. In addition to that, they should be stable and withstand several harsh reaction conditions such as high temperature, strong acid, strong alkaline, high salinity, extreme pH, tolerate high substrate/product concentrations and solvent tolerance. The methodology for prospecting highly stable enzymes is to prospect microorganisms from extreme habitats. The main difficulty is the propagation of potent microorganisms from extreme environment in laboratory conditions and most of the biocatalysts for industrial purpose are discovered through metagenomics (Yang et al., 2016). Thus, to accomplish all these qualities enzymes are nowadays tailor made by various enzyme engineering and stabilisation techniques for application in several fine chemical syntheses.

Modern bioengineering tools as well as advances in computational tools have revolutionised the field of enzyme engineering by designing different novel as well as improved enzymes suitable for different range of enzyme catalysed reactions during industrial processes. Biocatalysis is possibly to be the future of fine chemical production, and engineered and recombinant enzymes will be available in the future for most of the industrial and pharmaceutical processes. The immense advancement in this field has been made a reality by the fast development of different omics technologies, system biology, biochemistry, structural biology and other computational tools.

The intensifying interest for biocatalyst can be met in either by improving the catalytic power of the currently using enzymes or by bio-prospecting of novel enzymes. This review focuses on the current and emerging trends for enzyme technologies, and different strategies for creating novel enzyme biocatalysts.

2. Bio-prospecting of enzymes

Large number of enzymes possessing different activities is already discovered. But still it represents only a tiny fraction of the natural diversity available in nature. More than 99% of microbial diversity is also not possible to cultivate under laboratory conditions. Methods have been developed to prospect these extreme environmental niches and uncultivable microbes. The discovery of novel enzymes from such habitats would also involve the use of specialised techniques like microbial culture of uncultivable and high throughput screening methods for desired activities/properties (Wang et al., 2016).

2.1. Bio-prospecting from extremophiles

The genetic diversity of extreme environments of pH, temperature, alkalinity, salinity can be exploited to discover new and potent enzymes that are well suited for use in industrial processes and are relatively unexplored. The most important example is the discovery of biotechnologically important Taq DNA polymerase which is obtained from the extreme thermophilic bacterium *Thermus aquaticus* (Chien et al., 1976). The commercial market of this particular enzyme was about $500 million in 2009 (Adrio and Demain, 2014). Large number of extremophilic genomes has been sequenced by next-generation sequencing technologies and provides rich resources for prospecting of new enzymes with novel activities. The mechanism behind protein stability under extreme environmental conditions varies in different microbial species and level of adaption required for survival. For the acidophiles or alkaliphiles, the intracellular proteins are not tolerant at extreme pH since the intracellular pH is maintained at pH 5.0–6.0 and the cell membrane proteins are more tolerant to extreme pH. During industrial processes esterases has to function in solvent containing media and thus it should be solvent tolerant. The important functional characteristic that helps esterases to be stable in organic solvents include the presence of large number of negatively charged amino acid residues on their surface. This mechanism is also utilized by the halophiles to cope with high salt salinity, and hence, enzymes from such bacteria are expected to work in the presence of organic solvents. Similarly, cold active enzymes have tremendous applications in textile and detergent industry, fruit juice clarification, environmental bioremediation, etc., can be prospected from psychrophiles, which are normally adapted to function in extremely cold temperatures (Santiago et al., 2016). So detailed