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Recent advances in biocatalyst discovery, development and applications

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ABSTRACT

Enzymes catalyze a wide range of biotransformations and have a great potential as environmentally friendly alternatives to classical chemical catalysts in various industrial applications. Recently, advanced techniques and strategies in enzyme discovery and engineering have led to the significant expansion of the quantity and functional diversity of biocatalysts, which has further allowed broader uses of biocatalysts in new processes, especially those traditionally enabled only by chemical catalysts. Here we highlight some of these recent advances with the focus on new approaches in biocatalyst discovery and development, and discuss new applications of selected biocatalysts including transaminases, cytochrome P450s, and Baeyer–Villiger monooxygenases.

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1. Introduction

Enzymes carry out a wide array of chemical reactions essential for life and have increasingly been used as alternative catalysts in industrial processes such as the production of pharmaceuticals, agrochemicals, pesticides and insecticides.[1] Compared with chemical catalysts, the use of biocatalysts offers a number of distinct advantages such as high efficiency, high degree of selectivity, mild reaction conditions, and environmental friendliness.[2] These features have been driving enormous efforts in creating biocatalysts to fulfill consumer demands for new and higher quality products, industrial demand for improved efficiency, and society and government pressures for 'greener' technologies.

The huge potential of biocatalysts can be realized only if we can discover and develop suitable enzymes for a given industrial application. In the last few years, new approaches have been developed to discover new enzymes and new homologs to suit industrial processes. Generally, enzymes evolved for biological systems rarely perform optimally under conditions favorable for chemical synthesis such as high concentration of organic solvents and extreme conditions of pH, temperature and/or pressure.[3–5] However, the pool available for biocatalyst selection and development is primarily comprised of enzymes only from easily accessible and cultivable organisms, while about 99% of the host organisms on Earth are uncultivable and remain unexplored for discovery of novel biocatalysts.[6] Exploitation of this untouched resource will not only provide numerous unidentified enzymes from microorganisms residing in environmental conditions similar to those of industrial processes (pH, pressure, temperature, etc.), but also allow developing new processes.[7,8] Indeed, the efficiency of this approach was well demonstrated ten years ago.[9] In this study, >600 libraries were prepared with environmental DNAs of both cultivable and uncultivable microbes, and library screening led to the increase in number of total identified nitrilases by 7 times. Besides, exponential growth of new enzyme structures, enormous amount of -omics data, and rapid advances of bioinformatics tools together are revolutionizing our strategies in identifying and characterizing new enzymes from existing and new databases.[10–12]

Protein engineering technologies are often used to change and optimize enzyme traits for chemical reactions. Both rational design and directed evolution have been used to tailor enzyme properties.[13,14] Rational design requires thorough understanding of parental structure, catalytic mechanism, interactions, and even dynamics in order to identify mutations that would lead to desired enzyme properties, especially the high level of performance required for industrial applications. Such information is often lacking, particularly when new enzymes are used in biocatalyst development. Evolutionary approach that only requires functional expression in a recombinant host and a high throughput screening or selection is commonly used to improve enzyme fitness. It mimics Darwinian

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evolution process in laboratory settings and importantly on a significantly shorter time scale through iterative cycles of mutagenesis and accumulation of beneficial mutations.[15,16] Freed from natural constraints, computational protein design is another promising approach in developing biocatalysts. Very recently, the Hilvert group improved the activities of computationally designed biocatalysts to the level of natural enzymes by combining rational design and directed evolution approaches.[17–19] All of the above enabling techniques offer exciting new opportunities to develop biocatalysts for better and new applications.

In this review, we discuss recent advances in the discovery of enzymes as biocatalysts and their developments with new approaches. Over the past few years, a number of applications with new biocatalysts have been reported. Given space limit, we discuss only a few in this review, and refer readers to two recent excellent reviews for gaining additional information.[20,21]

2. New approaches in the discovery of novel biocatalysts

Historically, the discovery of enzymes is mostly based on the culture enrichment and screening of crude extracts from natural sources.[22,23] These approaches have led to the identification and characterization of a large number of biocatalysts that have been used in many applications. However, the cultivable microorganisms represent only <1% of the microbial diversity, thereby significantly limiting search spectrum for new enzymes.[24] With emergence of cultivation-independent techniques and increasing availability of (meta)genomics and enzyme structure information in public databases, metagenomics-based searching and big data mining are becoming feasible and useful in the discovery of new enzymes (Fig. 1).

2.1. Discovery of new enzymes by metagenomics-based approaches

Metagenomics-based approaches are comprised of extracting microbial DNAs directly from environmental samples, cloning into suitable expression vectors, transforming into easily cultivable bacteria, and isolating desirable clones for enzyme identification and characterization (Fig. 1). This strategy effectively lifts the constraint of enzyme host cultivability since genetic materials of both cultivable and uncultivable microbes are used.[6,25,26] Over billions of years, Nature has created, evolved, and selected a myriad of functional diversity of enzymes and their associated biochemical pathways to catalyze numerous biotransformations under diverse biological settings. Capitalizing on this rich diversity residing in the vast number of microbes will expectedly deliver novel and more suitable biocatalysts.

Discovery of novel biocatalysts from the metagenomic libraries generally relies on two strategies: function-based analysis and sequence-based screening.[27] Both strategies have led to the discovery of a number of enzymes in different studies, and we

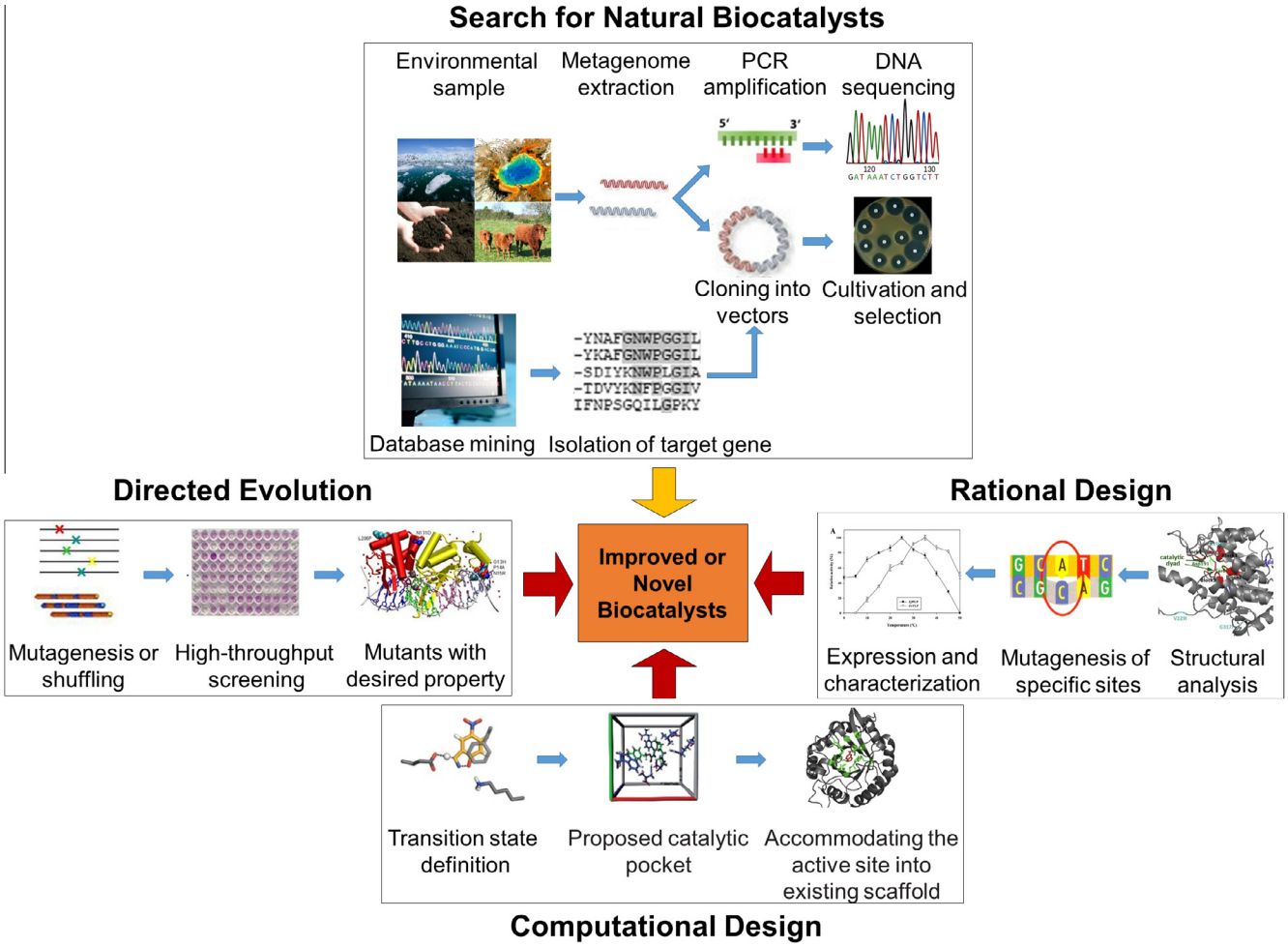

Figure 1. Overview of approaches for protein discovery and engineering. Yellow arrow indicates the approaches for the discovery of natural enzymes, and red arrows show three strategies of protein engineering. All together lead to new and improved biocatalysts for novel applications.

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