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Review

Directed evolution of biocatalytic processes

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Abstract

The benefits of applying biocatalysts to organic synthesis, such as their high chemo-, regio-, and enantio-specificity and selectivity, must be seriously considered, especially where chemical routes are unavailable, complex or prohibitively expensive. In cases where a potential biocatalytic route is not yet efficient enough to compete with chemical synthesis, directed evolution, and/or process engineering could be implemented for improvements. While directed evolution has demonstrated great potential to enhance enzyme properties, there will always be some aspects of biocatalytic processes that it does not address. Even where it can be successfully applied, the resources required for its implementation must currently be weighed against the feasibility of, and resources available for developing a chemical synthesis route. Here, we review the potential of combining directed evolution with process engineering, and recent developments to improve their implementation. Favourable targets for the directed evolution of new biocatalysts are the syntheses of highly complex molecules, especially where chemistry, metabolic engineering or recombineering provide a partial solution. We also review some of the recent advances in the application of these approaches alongside the directed evolution of biocatalysts.

Keywords: Directed evolution; Biocatalysis; Process; Metabolic engineering; Recombineering; Microscale

1. Directed enzyme evolution and biocatalytic processes

While enzymes are potentially a very useful complement to the increasing range of chemical catalysts for the synthesis of complex chiral molecules, the process conditions in an industrial catalytic reactor are rarely ideally suited to maintaining a highly active and long lasting enzyme. Natural enzymes have evolved over millions of years to operate in a specific environment and despite their wide diversity, natural, and industrial environments are usually significantly different (Table 1). In a recent paper, some of the ideal properties that would be required of an enzyme for an industrial process have been discussed [1] and it is clear that environments appropriate to deliver such properties are not easily found in nature. In addition, enzyme chemistry in nature is rarely the most useful to assist in the synthesis of complex industrial products. These factors make the application of enzymes for industrial synthesis difficult, in particular, where multiple (rather than singular) traits need to be satisfied to create the ideal industrial enzyme catalyst. Nevertheless, there are very strong reasons for attempting to overcome these barriers, such as the exquisite chemo-, regio-, and stereo-selective and specific properties of enzymes and their ability for effective catalysis in water, under mild conditions.

One method of overcoming some of the barriers to implementing enzymes in industry is the application of directed evolution, in which the amino acid sequence of an enzyme is iteratively altered until the enzyme functions in the desired manner. Changing a protein by directed evolution opens the possibilities for moving towards a variety of required properties. The most popular targets for directed enzyme evolution to date have been activity [2–4], substrate specificity [5–10], thermal and oxidative stability

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Table 1
Typical conditions in an industrial catalytic reactor

Molar concentrations of reactant/product
High salt concentrations
Temperature and pH set by reactant/product stability
Presence of gas–liquid interface
Heterogeneity

[11], enantio-selectivity or enantio-specificity ([12,13], pH range [14], and tolerance to solvent [15]. In many cases, directed evolution of an enzyme has enabled biocatalysis to reduce the number of steps required in an equivalent chemical or biocatalytic synthesis. Recent examples include the syntheses of 7-aminodesacetoxycephalosporanic acid (7-ADCA), shown in Fig. 1 [16], (*S*)-ketoprofen [17], and both natural and unnatural sugar-1-phosphates for access to glycoconjugates [18]. It is unlikely that all biocatalytic process targets can be achieved through directed evolution, although other techniques in which the process environment is changed may prove useful instead.

While directed evolution is a powerful method to overcome some of the limitations of biocatalysts, it can take considerable time to implement. On the other hand, improvements can be made through process techniques more rapidly, although they may be rather more modest (Fig. 2). Understanding this trade-off will be key to the future rapid implementation of processes in this area. Ultimately, a combination of approaches will enable better implementation of effective biocatalytic processes. Some of the recent developments in these techniques are summarised in Fig. 3.

There are several other major aspects of biocatalytic processes that still largely need to be addressed by directed evolution. For example, the concentrations of substrates

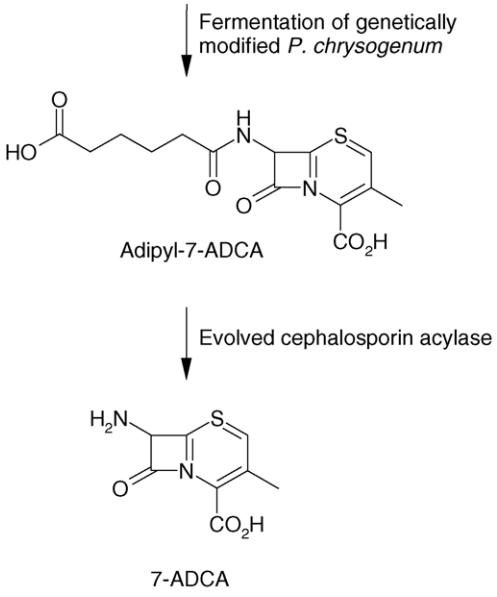

Fig. 1. Synthesis of 7-aminodesacetoxycephalosporanic acid (7-ADCA) from adipyl-7-aminodesacetoxycephalosporanic acid (adipyl-7-ADCA) using an evolved cephalosporin acylase.

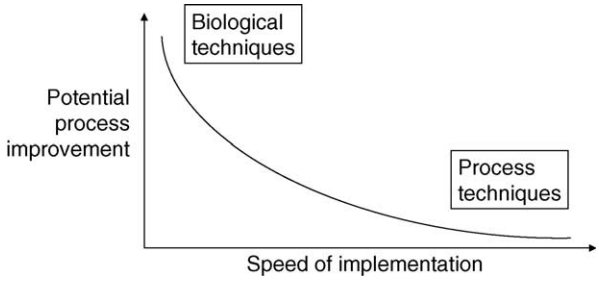

Fig. 2. Potential trade-off between the rapid implementation of process techniques and high process improvement from biological techniques such as directed evolution.

used in industrial chemical reactions are usually as high as possible to reduce costs, speed up reactions, and to improve product purification. The molar concentrations used in industrial chemical processes would frequently lead to toxic or inhibitory effects on a biocatalyst, or the accumulation of unwanted by-products. Directed evolution has the potential to reduce the effects of product inhibition [19] or by-product formation, and permit high process-intensity biocatalysis. Another factor to consider is the physico-chemical properties, including solubility and stability, of the substrates and products, which often dictate the conditions to be used in the bioreactor. These may be at odds with the enzyme (e.g. different pH optimum) and directed evolution may be used to alter the enzyme properties to match the conditions required in the reactor [20]. Finally, the requirement of redox enzymes for cofactors presents a major problem for using them in industrial biocatalysis as the cost of cofactor is prohibitive. While options are available for recycling cofactors [21], directed evolution has been used previously to alter the cofactor requirement of *Pseudomonas putida* P450 from NADH to a much cheaper chemical alternative, hydrogen peroxide [22].

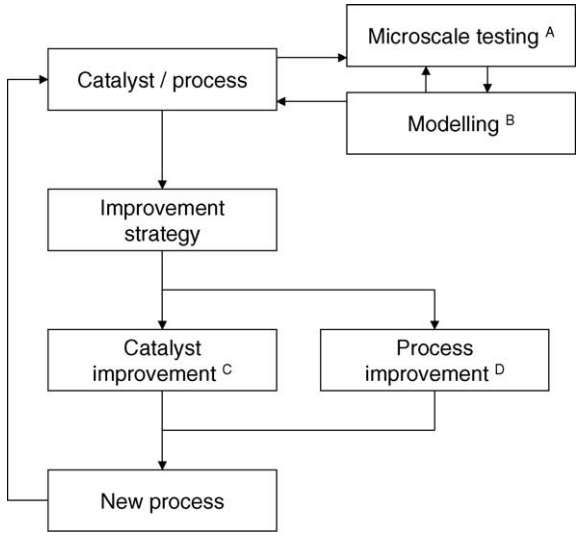

Fig. 3. Recent developments to assist in the rapid implementation of effective high-intensity biocatalytic reactions. A: [25], B: [20], C: [44,92,23], D: [93–95].