Developing strategies for engineering efficient biocatalysts for the industrial production of chiral compounds

The market of chiral pharmaceuticals and of other fine chemicals for the fragrance and plant-protecting industries is a global multi-billion dollar business which is steadily growing. In general, only one of the enantiomers, the (R) or the mirror image (S)-isomer, is biologically active. This means that economically and ecologically viable methods for accessing either one of the enantiomers are needed, preferably in a catalytic process. Three options are basically possible, chiral transition metal catalysis, organocatalysis or biocatalysis, all suffering from various disadvantages which have been delineated elsewhere.

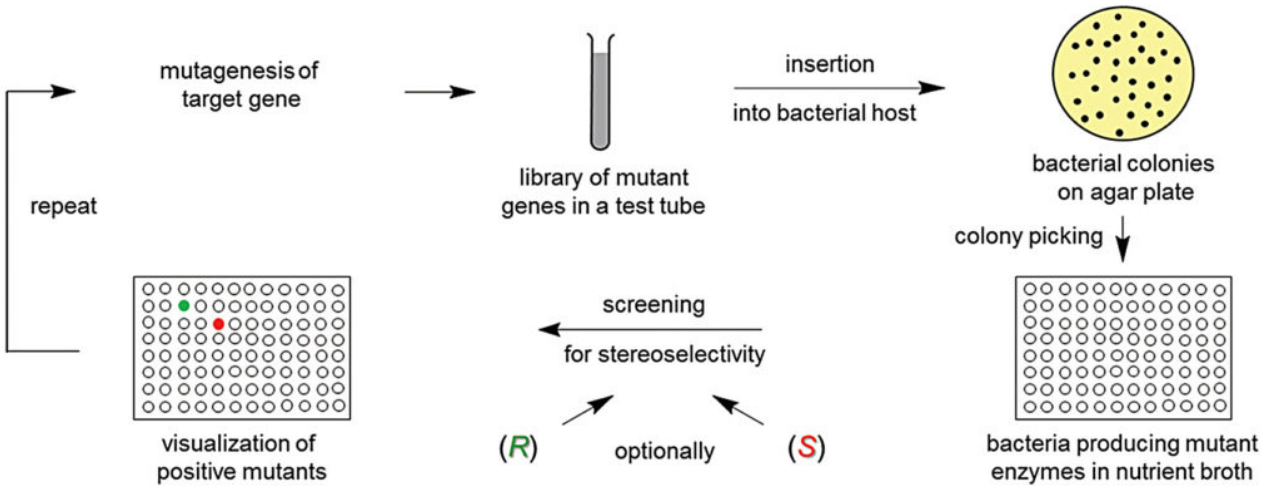

Fig. 1. The concept of directed evolution of stereoselective enzymes as catalysts in organic chemistry and biotechnology.

In the case of biocatalysis, the use of naturally occurring (wildtype) enzymes often leads to poor or wrong enantioselectivity and/or insufficient activity. Two decades ago we proposed and implemented experimentally the concept of directed evolution of stereoselective enzymes as catalysts for asymmetric transformations in organic chemistry and/or biotechnology (Fig. 1). It is based on recursive cycles of gene mutagenesis, expression and screening, each round building up evolutionary pressure for the formation of either the (R)- or the (S)-enantiomer on an optional basis. In the early days we and subsequently other groups used error-prone polymerase chain reaction (epPCR, a shotgun method), saturation mutagenesis (focused mutant libraries) and/or DNA shuffling as the genetic techniques. However, for wide industrial applications, methodology development was necessary for fast and reliable biocatalyst creation.

Originally large mutant libraries had to be screened for enantioselectivity, which is the labor-intensive step of the overall process. Therefore, we embarked on developing highest-quality mutant libraries based on the use of structure- and bioinformatics-guided saturation mutagenesis, if necessary in an iterative manner. Statistical analysis showed that oversampling for 95% library coverage rapidly reaches astronomical numbers even when medium-large randomization sites are targeted. Therefore, we introduced the use of reduced amino acid alphabets, meaning the employment of less than the usual 20 canonical amino acids, e.g., 12, 8 or 5. Most recently, we showed that even one, two or three amino acids as combinatorial building blocks provide excellent results. But where is the optimum?

In the highlighted study, single code saturation mutagenesis (a single building block) is compared with double code saturation mutagenesis (two building blocks). We demonstrate that the latter is distinctly more efficient, and identify the reasons for this trend using an enantioselective epoxide hydrolase as the model process. Mutants for both enantiomers were readily evolved. Moreover, the final results regarding the degree of enantioselectivity of active mutants are compared with the use of triple code saturation mutagenesis in the same asymmetric transformation, which proved to be superior.

In conclusion, directed evolution of enantioselective enzymes based on the use of minimal libraries generated by saturation mutagenesis in which only two or preferentially three amino acids are used as combinatorial building blocks constitutes a prolific source of efficient catalysts. We applied this approach in the synthesis of intermediates needed for the production of chiral pharmaceuticals.

Zhoutong Sun [1,2,3], ***Richard Lonsdale*** [1,2], ***Guangyue Li*** [1,2], ***Manfred T. Reetz*** [1,2]
[1]*Chemistry Department, Philipps-University, Marburg, Germany*
[2]*Max-Planck-Institut für Kohlenforschung, 45470 Mülheim, Germany*
[3]*Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences*

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