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Bacterial surface display as a technology platform for high-throughput screening of enzyme library: A cellulase case

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Directed evolution is increasingly being used to improve biocatalysts. As more powerful combinatorial mutagenesis methods (e.g., combinatorial cassette mutagenesis, StEP and DNA shuffling (1) become available, designing selection or screening strategies becomes the most critical step in the successful exploitation of generated molecular diversity. Screening can be based on chromogenic substrates or easily observed colony phenotypes, but the most direct methods of screening and selection are to link improved enzyme activity to the survival or growth rates of cells. Examples of this method include selection on plates containing increasing antibiotic concentrations and complementation selection using auxotrophs. Unfortunately, these selection procedures are designed for specific enzyme activities, making the generalized identification of improved enzyme variants difficult. In addition, these methods work only if the activity of the target enzyme does not interfere with cellular metabolism and can be distinguished from the background of all other cell reaction.

An alternative selection method is to display libraries of mutated proteins on phage or microbial cell surfaces and then select mutant enzymes having desirable properties (2, 3). Enzymes displayed on phage, for example, may be screened for improved affinity for desired substrates and the corresponding clones selected by panning techniques. Recently, a library of surface protease OmpT was displayed and clones showing improved substrate affinity were isolated by flow cytometry. In our study, we have used the ice nucleation protein (Inp)-based bacterial surface display system (4) to selectively screen enzyme libraries for improved catalytic activity.

Our model enzyme was carboxymethylcellulase (CMCase). Because CMC (carboxymethylcellulose) is a high-molecular-weight polymer, it is not transported into cells. Thus, cells that display CMCase on their surfaces only hydrolyze CMC in agar plates. Cell colonies that hydrolyze CMC can be easily recognized because they are surrounded by a clear halo after staining with Congo-Red. Because *E. coli* cells displaying CMCases form tiny colonies on M9 minimal medium containing CMC as the sole carbon source, we reasoned that altered growth rates of transformants would be correlated with the activities of displayed

CMCase variants. Thus, by selecting rapidly growing colonies, only the cells containing improved CMCase variants would be isolated and checked further, obviating laborious random plating and assay procedures. In this report, we describe a growth-based direct screening method for the identification of improved CMCases based on the display of an enzyme library on the bacterial cell surface (5). While most directed evolution experiments have attempted to improve binding of non-natural substrates and/or enzyme stability in non-natural environments (2), we have shown here that overall catalytic efficiency of cellulase towards its natural substrate (cellulose) can be improved under physiological conditions.

In conclusion, this surface display of hydrolytic industrial enzyme libraries could provide a high-throughput screening environment according to the ability of the cells to grow on non-utilizable substrates as described here. The bacterial surface display method would be a technology platform for selection of improved enzymes produced by directed evolution, if Inp-enzyme fusion variants, for example, were functionally displayed. In addition, enzymes such as proteases and lipases, which are toxic to the cells when expressed in the cytosol, can be selected for improved characteristics using these techniques.

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