In vitro evolution of a beta-glucosidase aiming at cellulose hydrolysis for production of second generation ethanol

Rozenberg, R.; Marana, S.R.
Instituto de Química – Departamento de Bioquímica - USP

A digestive beta-glucosidase from the larvae of the fall armyworm *Spodoptera frugiperda* (Sfbgli; AF052729), an enzyme naturally active upon cellobiose, was submitted to a directed evolution protocol in order to pick up mutants presenting higher specificity for this disaccharide. Therefore, a plasmid coding for Sfbgli was submitted to random mutagenesis using both XL1-Red *E. coli* and the GenoMorph II EZClone kit (Stratagene), in conditions of low and medium mutation rates (average of one and two residues mutated per enzyme, respectively). The libraries were introduced in NovaBlue(DE3) *E. coli* (Novagen) and transformed colonies were induced to produce Sfbgli in 96 deep wells. Also in those plates colonies were disrupted and mutant Sfbgli were screened based on the rate of enzyme activity upon the artificial substrate 4-nitrophenyl-beta-D-glucoside (pNPG) over that of cellobiose. Four mutants presenting increased specificity for cellobiose were obtained. Interestingly, for three of them the decrease of the activity upon pNPG was higher than upon cellobiose, possibly due to epistatic robustness of the activity upon the natural substrate. In addition one mutant exhibited increased activity upon cellobiose, whereas the activity upon pNPG was reduced. We are in the process of sequencing the plasmids coding for these mutants, for further studies on enzyme kinetics and possibly for new rounds of selection. The mutants selected may increase our understanding on the structure-function relation of beta-glucosidases, as well as these evolved Sfbgli exhibiting higher specificity for cellobiose may compose enzymatic cocktails for cellulose hydrolysis.

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