# STUDY OF THE MOLECULAR BASIS FOR THE SUBSTRATE SPECIFICITY IN A b-GLYCOSIDASE BY USING DIRECTED EVOLUTION

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β-glycosidases from Glycoside Hydrolase family 1 (GH1) play diverse and important roles in living organisms. They remove monosaccharides from the nonreducing end of di-and/or oligosaccharides, present the same tertiary structure ($(\beta/\alpha)_8$ barrel), are configuration-retaining enzymes and their catalytic activity depends on two glutamic acid residues. In order to study the molecular basis for the substrate specificity of these enzymes, a digestive β-glycosidase from *Spodoptera frugiperda* (Sfβgly50) was submitted to directed evolution. A library of random mutants of Sfβgly50 was generated using the mutator *E. coli* strain XL1-Red. This library was used in a high-throughput screening based on the Sfβgly50 relative activity upon two different substrates (*p*-nitrophenyl-β-fucoside and *p*-nitrophenyl-β-glucoside). From 4032 screened colonies, 40 were selected as candidates to produce mutant Sfβgly50. These selected colonies were further characterized confirming that 14 of them produced mutant Sfβgly50 presenting a substrate preference which was significantly different from the wild-type Sfβgly50. Vectors coding these Sfβgly50 mutants were sequenced showing that all them contained single mutations, which were mapped outside the active site in a homology model of the Sfβgly50 spatial structure. Furthermore, none of them had been previously associated with the modulation of the substrate specificity in β-glycosidases. These 14 mutant Sfβgly50 were produced in *E. coli* BL21-DE3 and purified using affinity chromatography. Effects of these mutations on the Sfβgly50 specificity were characterized by determining kinetic ($K_m$ and $k_{cat}$) and thermodynamic parameters ($\Delta\Delta G^{\ddagger}$) for the hydrolysis of *p*-nitrophenyl-β-fucoside and *p*-nitrophenyl-β-glucoside. Concluding, it is proposed that these mutated residues affect the substrate specificity through connection networks linking them to Sfβgly50 active site.

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