CONSTRUCTION, EXPRESSION AND CHARACTERIZATION OF SITE-DIRECTED MUTANTS OF A DIGESTIVE LYSOZYME

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Digestive lysozymes are found in some insect groups, particularly in dipterans that feed on decomposing material. These enzymes are classified in the family 22 of the glycoside hydrolases and share common properties with lysozymes *c*. However, digestive lysozymes have acidic pH optimum and low substrate affinity at high ionic strength. In order to understand the basis of these properties, a digestive lysozyme (MdL1) from *Musca domestica* (housefly) was submitted to site-directed mutagenesis using an "overlapping primers" strategy. Based on that, four superficial residues (E11, R36, D79, D84 and K109) were replaced generating the single mutants E11Q, R36A, D79N, D84N, K109A and the triple mutant E11Q-D79N-D84N. Following that, segments coding mutant MdL1 were cloned in the vector pPic9. After confirmation that inserts were placed in the correct reading frame, pPic9 vectors coding the MdL1 mutants were used to transform *Pichia pastoris* GS115. Transformed colonies were induced using 1% methanol in BMM broth. Considering that recombinant proteins coded by the vector pPic9 are secreted by *P*. *pastoris* GS115, lysozyme activity upon supensions of *Micrococcus lysodeikiticus* (1 mg/mL) was detected on samples of the induction medium. The most productive colonies were chosen to produce the mutants E11Q, R36A, D79N, D84N, K109A and E11Q-D79N-D84N in large scale conditions. After that, samples containing these enzymes were concentrated using reverse-dialysis and the pH effect on their activities was determined at four ionic strengths (0.02, 0.07, 0.1 and 0.2). Mutant MdL1 presented low activity, but no significant pH optimum alteration was detected. Concluding, the production of these mutant MdL1 was well succeeded and their affinity for *M. lysodeikiticus* will be determined.

Keywords: lysozyme, pH optimum, substrate affinity

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