Telli, E.P.¹; Weiss, P.E.¹; Miletti, L.C.¹ and Magalhães, M.L.B.¹ Directed evolution of *Bacillus polymyxa beta glucosidase*. Universidade do Estado de Santa Catarina - Mestrado do Programa Multicêntrico em Bioquímica e Biologia Molecular, Lages, 2015.

Abstract

First generation ethanol production is the main process for bioethanol production and utilizes the sucrose present in sugar cane as raw material for the fermentation process, discarding two thirds of the biomass in the form of lignocellulosic residues. Second generation bioethanol production is a method to obtain ethanol from lignocellulosic biomass, but this method is still inefficient and costly due to the inefficiency of the enzymes involved in the process. Identification of a new beta-glucosidase (BGLA) (involved in cellulose hydrolysis process) with high catalytic efficiency for use in second generation ethanol production process would represent an important advance for bioethanol production. This project aims to identify mutants of the enzyme BGLA from *Bacillus polymyxa* with higher catalytic efficiency as compared to wild type through directed evolution techniques. A BGLA genomic library was produced through random mutation in seven residues (Q20, H121, N165, Y296, W398, W406 and E405) present in the active site of the enzyme. These residues that make direct contact with the gluconate inhibitor were randomly mutated by the megawhop technique. Selection of mutant enzymes was performed by transformation of the genomic library into *E. coli* Rosetta Gammi cells following growth on M9 minimal media containing cellobiose as the sole carbon source, appropriate antibiotics and IPTG. Selected colonies were subjected to sequencing. The catalytic efficiency ($V_{max} / K_m$) of each mutant will be determined by measuring their kinetic parameters ($K_m$ and $V_{max}$). The enzymes with catalytic efficiency higher than wild-type will be chosen to perform detailed enzymatic analysis.

Keywords: BGLA, catalytic efficiency, directed evolution, mutagenesis.

¹ Universidade do Estado de Santa Catarina.