Cloning and Heterologous Expression of the *Aspergillus niger* Cellulases Genes into *Kluyveromyces marxianus* UFV-3

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The second generation of ethanol is an alternative bioenergy source considering the oil world-wide dependence. The use of the sugar cane bagasse as biomass can increase an average 30% of the ethanol production without extending the sugar cane cultivated area. However, the obtaining of cellulosic ethanol depends on the reduction of the costs with the enzymatic hydrolysis process of the fiber to release nom-complex sugars to be fermented by yeast cells. One promising strategy for developing this process is the simultaneous saccharification and fermentation (SSF) by using recombinant fermenting yeasts expressing the cellulolytics enzymes. *Kluyveromyces marxianus* is thermotolerant yeast that is able to assimilate cellobiose as carbon source, to ferment pentoses and hexoses, besides that, presents an efficient secretion system. These characteristics become this yeast a potential organism in SSF processes. In this work the plasmid pKLAC1 (New England Biolabs®), was modified by the insertion of the *Kluyveromyces lactis* ADH2 promoter in order to allow the constitutive expression of heterologous proteins in the yeast *K. marxianus*. Furthermore we cloned *Aspergillus niger* cellulase genes *eng*1 (endo-ß-1,4-glucanase), and *cbh*A (cellobiohydrolase) into this modified vector under the ADH2 promoter control. The restriction sites used were *Xho*I and *Not*I (*eng*1), and *Sal*I and *Not*I (*cbh*A). These constructions were sequenced and applied to transform *K. marxianus* UFV3 strain. The heterologous expressions of the cellulolytic enzymes are in current analysis in our laboratory.

Key words: cellulosic ethanol, cellulases, *Kluyveromyces marxianus*
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