

## **Cloning and Initial Characterization of Cellulolytic Enzymes Selected in a Metagenomic Approach**

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### **Abstract**

**Introduction:** Fossil fuels are a limited source of energy that in its current usage causes harmful effects on the environment after its combustion. A possible alternative to fossil fuels is biofuels derived from non-food cellulosic plants and woods. A large amount of bagasse waste rich in cellulose is produced by the sugar industry. Cellulose is hydrolyzed by microbial cellulases into various forms of sugar that can be fermented into ethanol or other biofuels. Although some commercially cellulases are available, industry still seeks improvement in catalysis rate and sugar yield. We have aimed to isolate cellulases with greater potential usage in the biofuel industry using a metagenome library constructed from the gut of *Capra hircus*. **Material and Methods:** The libraries were prepared by shearing DNA into fragments that were cloned into bacterial vectors and screened for cellulolytic activities. The most active cellulolytic colonies were sequenced and genes were found using bioinformatics tools. Genes that coded for proteins with more than 100 aminoacids and had sequence similarities to glycosyltransferases and cellulases were selected. Primer design, amplification and cloning into expression vectors followed. The program Modeller 9v10 was used to build a 3D homology model for one of the proteins that presented a homologous structure available in PDB (2JJM). **Results and Discussion:** Selection criteria yielded nine novel genes that were amplified and cloned into bacteria plasmid vectors. We are working on the expression of these genes for active cellulolytic activities. The active site of the homology model has three residues (Lys211, Glu282, Glu290) that are strictly conserved among other member of the GT4 family of glycosyltransferases, indicating catalytic capability. **Conclusion:** We have selected, cloned and modeled putative genes/enzymes that have potential application in the biofuel industry. Our future aims include expression and purification of these proteins for enzymatic and crystallographic studies.

**Keywords:** Biofuel, Metagenome, Cellulose degradation, Enzymatic activity, Protein structure

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