Cloning, expression and structural modeling of a putative cellulolytic enzyme selected in the metagenomic library of *Capra hircus* rumen microbiota

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INTRODUCTION: Biofuels produced from lignocellulosic biomass are a possible alternative to fossil fuels since this substrate is largely available and generates benefits to the environment. Diverse enzymes can efficiently degrade lignocellulosic biomass into various forms of carbohydrates, which can then be fermented into ethanol and other biofuels. Metagenomic screening studies are a source of new enzymes that could play a role in the production of 2nd generation bioethanol. **OBJECTIVES:** Cloning, expression and structural analysis of a novel cellulolytic enzyme selected from the *Capra hircus* gut metagenome and its structural characterization. **MATERIAL AND METHODS:** An open reading frame (ORF) with homology to reported cellulases was selected in the metagenome positive clones with cellulose activity. This ORF was cloned into a plasmid pET28a that was used to transform *E. coli* BL21 cells. Expression tests were performed varying temperature, time and induction strategy (auto or IPTG). Inclusion bodies solubilization was tested using increasing concentrations of urea. A comparative structural model was constructed by protein modeling servers. The resulting models were evaluated and the best models were selected as templates for modeling using the MODELLER program. **RESULTS AND DISCUSSION:** There was an excellent structural conservation between the predicted model and protein members of the GT-B family. A comparison of the active site architecture of the protein with other glycosyltransferases revealed conserved positive charged amino acids that stabilize the substrate's β-phosphate. The protein has shown expression in different IPTG concentrations at 25 °C, 28 °C and 37 °C. Most of the protein became soluble at 8 M urea solution. Judging by the SDS-PAGE band profile, the resulting protein may be truncated. **CONCLUSIONS:** A new protein with putative cellulase-related activity has been cloned, expressed and solubilized. New expression tests should be performed before purification trials proceed in order to avoid inclusion bodies.

Keywords: Metagenome, Glycosyltransferase, Heterologous Expression

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