Cloning, Expression and Solubilization of a Putative Cellulolytic/Xylanolytic Enzyme Identified in the *Capra hircus* Gut Metagenome

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INTRODUCTION: Fossil fuels are a limited source of energy and their massive use contributes to its rapid depletion and environmental pollution, thus searching for alternative energy sources like biofuels are quite attractive. Second-generation bioethanol produced from lignocellulosic biomass of bagasse discarded by sugar industries has gained attention recently. Microbial enzymes perform hydrolysis of cellulose and hemicellulose present in this biomass to release carbohydrates that are ultimately fermented to produce ethanol. **OBJECTIVES:** Cloning, expression and solubilization of a putative cellulolytic/xylanolytic enzyme, which was selected from the metagenome of *Capra hircus* rumen microbiota. **MATERIAL AND METHODS:** The open reading frame (ORF) sequence for the enzyme was obtained from a metagenomic clone of *C. hircus* gut microbiota screened for cellulolytic and xylanolytic activity. The selected ORF was amplified by PCR, cloned in vector (*pGEM-T* Easy), subcloned into an expression vector (*pET28a*) and expressed in *E. coli* strain BL21 (DE3) pLysE. Due to the formation of inclusion bodies, expression tests were performed using different temperatures and IPTG concentration. Protein solubilization was obtained only by using different concentrations of urea**. RESULTS AND DISCUSSION:** The protein expression for the selected ORF was achieved in the form of inclusion bodies. Protein was solubilized using 2 M, 4 M and 8 M urea. A greater amount of solubilized pure protein was obtained with 8 M urea. The solubilization of the protein in low concentrations of urea (2 M) is an indicative that the inclusion bodies were small and low density, with weak interactions between partially folded regions of the protein. **CONCLUSIONS:** The cloning, expression and purification of the protein from solubilized inclusion bodies was successful. Further work on the solubilization of inclusion bodies for a posterior refolding step and/or other expression tests with changes in the bacterial strain is underway to obtain native soluble.

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