

## Recombinant Expression and Characterization of a Xylanase From *Leucoagaricus gongylophorus*

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**INTRODUCTION:** *Leucoagaricus gongylophorus* is the mutualist fungus of leaf-cutting ants belonging to the genera *Atta* and *Acromyrmex*. The mutualism between the ant species and the fungus has been the subject of many studies from the point of view of evolution, as well as providing digestive enzymes by the fungus to the ants being one remarkable aspect of the symbiosis. **OBJECTIVES:** The aim of this study was to express and characterize a xylanase (LgXyn2) from *L. gongylophorus*. **MATERIAL AND METHODS:** Oligonucleotides based on the gene of *L. gongylophorus* xylanase nucleotide sequence (GenBank:EF208066) were designed. cDNA obtained from the mRNA of the mycelium was used as template to amplification of the xylanase ORF. Amplified DNA fragment was cloned into the pPICZα-A vector (pPICZα-A-LgXyn2-His), and the vector was transformed in *Pichia pastoris* cells of three different strains: GS115, KM71 and X-33. The transformants cells were selected on agar containing zeocin. Expression experiments were accompanied by SDS-PAGE, westernblotting and enzymatic activity using the method acid 3,5- dinitrosalicílico (ADNS) with 1% xylan in McIlvaine buffer pH 5.0 as substrate. Once the best expressing clone/strain was identified it was used for large scale expression. The xylanase was purified in nickel resin and characterized. The optimum pH was determined using different buffers (McIlvaine buffer pH 2.2 to 8.0 and glycine-NaOH buffer for 9.0 to 12.0). The incubation temperature (20 °C to 80°C) was modified to verify the influence in the activity. **RESULTS AND DISCUSSION:** The best strain for LgXyn2 express was GS115 with induction by 1% methanol. LgXyn2 showed an optimum temperature of about 20 °C and the optimum pH around 5.0. **CONCLUSION:** The xylanase from *L. gongylophorus* was expressed in *P. pastoris* is soluble and active form. The optimum pH value for the LgXyn2 gives an interesting feature for use in the food animal industry.

**Keyword:** *Leucoagaricus gongylophorus*, xylanase, heterologous expression, *Pichia pastoris*.

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