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A HIGH-ADDED VALUE PRODUCT FROM TOMATO POMACE CONVERSION: α-L-ARABINOFURANOSIDASE FROM *Pleurotus ostreatus* FOR LIGNOCELLULOSE CONVERSION

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Abstract

2nd generation bioethanol produced from lignocellulose is one of the best alternatives to fossil fuels to achieve the targets established by Directive 2009/28/CE. Its advantages include low net greenhouse gas emissions, abundance and geographically more even distribution of lignocellulose raw materials, minimization of the potential food-versus-fuel conflict. (Hemi)cellulases are needed for lignocellulose hydrolysis, leading to the production of fermentable sugars. The major cost driving factor in lignocellulose-to-ethanol process is the high cost of (hemi)cellulolytic enzymes, so that reduction of their production costs and improvement of their performances are required to improve process competitiveness.

Solid state fermentation (SSF) is an economic and environmentally friendly alternative to conventional process for industrially relevant enzymes production. On the other hand, white-rot fungi (e.g. *Pleurotus ostreatus*) represent the most appropriate microorganisms for producing enzymes through SSF because of the similarity between their natural environment and the conditions in which SSF processes are carried out.

In this work, an α-L-arabinofuranosidase produced by the fungus Pleurotus ostreatus (PoAbf) during SSF on tomato pomace was identified, and the corresponding gene and cDNA were cloned and sequenced. The amino acid sequence similarity to the other α-L-arabinofuranosidases indicated that the enzyme can be classified as a family 51 glycoside hydrolase. Heterologous recombinant expression of PoAbf was carried out in the yeasts *Pichia pastoris* and *Kluyveromyces lactis* achieving the highest production level of the secreted enzyme (180 mg L-1) in the former host. rPoAbf produced in *P. pastoris* was purified and characterized. It is a glycosylated monomer with a molecular weight of 81,500 Da. Mass spectrometry analyses led to the localization of a single O-glycosylation site. The enzyme is highly specific for α-L-arabinofuranosyl linkages, it followed Michaelis–Menten kinetics with a KM of 0.64 mM and a kcat of 3010 min-1 when assayed with p-nitrophenyl α-L-arabinofuranoside, and exhibited an optimum pH of 5.0 and an optimal temperature of 40 °C. PoAbf is also able to hydrolyze natural substrates, besides some tested oligosaccharides. It is worth noting that the enzyme shows a very high stability in a broad range of pH. The durable activity showed by rPoAbf in comparison to the other α-L-arabinofuranosidases enhances its potential for biotechnological applications and increases interest in elucidating the molecular bases of its peculiar properties.

Design of PoAbf mutants was carried out in order to deepen both the role of its catalytic residues and the effect of glycosylation on enzyme stability.