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## ACCEPTED MANUSCRIPT

# Heterologous expression and biochemical characterization of a novel thermostable *Sclerotinia sclerotiorum* GH45 endoglucanase in *Pichia pastoris*

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#### ABSTRACT

Enzymatic saccharification of lignocellulosic biomass has been widely studied. Mainly endoglucanases were found to be a prerequisite for the quick initial biomass liquefaction. In the present study, Pichia pastoris was used as a host for the heterologous expression of a Sclerotinia sclerotiorum GH45 endoglucanase, Endo2. The recombinant plasmid pPICZaA was used to transform Pichia pastoris. Pichia culture supernatants expressing the recombinant *Endo2* (r*Endo2*) were used for the purification and biochemical characterization of this enzyme. Therefore, rEndo2 was purified 6.7 fold to homogeneity with 34% yield and gave 19 U/mg specific activity. It also showed maximum activity at pH 7.0 and 60°C (against pH 5.0 and 50°C for the native enzyme) and was thermostable at relatively high temperatures. Furthermore, rEndo2 retained its activity in a wide pH range (from 5 to 8). Besides, the recombinant endoglucanase was produced as an active 47 kDa enzyme. This molecular weight differs from the one of the native enzyme (34 kDa), which suggested a potential glycosylation of the recombinant enzyme. Moreover, rEndo2 was able to produce fermentable sugars after enzymatic assay on various cellulosic substrates with an interesting yield. Therefore, all these features offer prospects for large-scale production and industrial application of the recombinant endoglucanase.

Keywords: Pichia pastoris, cloning, endoglucanase.

#### 1. Introduction

Lignocellulosic biomass conversion to energy and/or high-value products provides an attractive alternative to today's petrol-based production of chemicals and transportation fuels [1]. The main component of the lignocellulosic biomass is cellulose; a linear polymer of  $\beta$ -1,4 linked glucose units. Its saccharification into glucose monomers requires the coordinated action of mainly three complementary enzymes: endoglucanases, exoglucanases and  $\beta$ -glucosidase

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