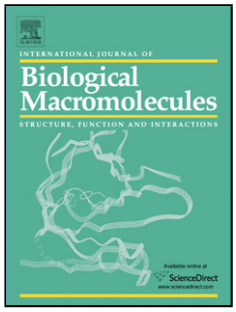

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Heterologous expression and biochemical characterization of a novel thermostable *Sclerotinia sclerotiorum* GH45 endoglucanase in *Pichia pastoris*

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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, *Endo*2. The recombinant plasmid *p*PICZαA was used to transform *Pichia pastoris*. *Pichia* culture supernatants expressing the recombinant *Endo*2 (r*Endo*2) were used for the purification and biochemical characterization of this enzyme. Therefore, r*Endo*2 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, r*Endo*2 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, r*Endo2* 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 β-1,4 linked glucose units. Its saccharification into glucose monomers requires the coordinated action of mainly three complementary enzymes: endoglucanases, exoglucanases and β-glucosidase