Heterologous Expression of Xyloglucan-specific Endo-β-1,4-gluconase (GH12) from *Aspergillus terreus* in *A. nidulans*

Vitcosque, G.L.¹; Ribeiro, L.F.C.¹; de Lucas, R.C.¹; Jorge, J.A.²; Polizeli, M.L.T.M.²

¹ Dep. de Bioquímica e Imunologia, FMRP, USP, SP, Brasil; ² Dep. de Biologia, FFCLRP, USP, SP, Brasil.

Xyloglucan is the predominant hemicellulose in primary cell walls of higher plants. Agricultural and forest waste products are abundant and low-cost biomass sources are used in renewable energy. Hydrolysis of a major biomass component, hemicellulose, is accomplished by the action of several enzymes. The use of recombinant DNA technology for the production of lignocellulolytic enzymes permits large-scale expression, optimization in the purification process and reduces the saccharification costs during generation of bioethanol. The aim of this work was the heterologous expression in *Aspergillus nidulans* of a xyloglucan-specific endo-β-1,4-gluconase (GH12) isolated from the whole-genome DNA sequence of *A. terreus* using pEXPYR, a vector designed for proteins overexpression in filamentous fungi, which directs proteins towards the extracellular medium in *A. nidulans* host strain. The coding sequence for the gene xyloendoglucanase (XEG1) was amplified by the polymerase chain reaction (PCR) using primers which include the restrictions sites for NotI and XbaI. After cloning of the amplified XEG1 fragment into pEXPYR and confirmation by nucleotide sequencing, the construction was transformed into *A. nidulans*. Positive transformants were isolated by their ability to grow in minimal medium (MM) missing uracil, uridine and 5-fluorotic acid, but containing 1g/L pyridoxine as a selection marker. Protein expression was carried out in MM supplemented with 5% maltose as inducer at 37°C under static and agitation conditions. XEG1 production was monitored by culture supernatant analysis by SDS-PAGE and the hydrolytic activity against xyloglucan was measured by reducing sugar releasing using the dinitrosalicylic acid reagent (DNS). XEG1 was expressed after 48h of incubation at 37°C in both culture conditions before mentioned. Catalytic activity against 0,5% xyloglucan from tamarind was 0,18 Ui/mL, under 15 min of incubation, pH 6,0 at 60°C. Thus, the cloning, heterologous expression and Xyloglucan-specific Endo-β-1,4-gluconase secretion from *A. terreus* was successfully executed in this research.

*Palavra chave:* heterologous expression, xyloendoglucanase, filamentous fungi, bioethanol.

*Patrocínio:* FAPESP, CAPES and CNPq.