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ABSTRACT BOOK



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Biotechnology / Synthetic Biology / Systems Biology - Part II

OVERPRODUCTION OF A TRICHODERMA HARZIANUM CHITINASE AND ANALYSIS OF ITS BIOTECHNOLOGICAL POTENTIAL TO PRODUCE CHITOOOLIGOSACCHARIDES

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Backgrounds

Chitooligosaccharides (COS) are β -(1,4)-linked oligomers of N-acetyl-glucosamine (GlcNAc) and glucosamine (GlcN) formed by chemical or enzymatic hydrolysis of chitosan or chitin. The growing biotechnological interest of COS in fields such as food or health increases the demand of the producing enzymes as well as their characterization and functional improvement.

Objectives

Express a chitinase of 42 kDa from *Trichoderma harzianum* in a heterologous system, obtain protein levels compatible with its crystallization for the future protein structural resolution and evaluate the ability of the recombinant protein to produce COS.

Methods

The chitinase *gene* cDNA from *T. harzianum* was expressed in *Pichia pastoris* using a restriction-free cloning strategy, production of heterologous protein was analysed and escalated up to a 5 L fermenter level. Recombinant protein was purified and some crystals were obtained which allows undertake the protein structural resolution. Synthesis of oligosaccharides from different substrates were evaluated and optimized using the recombinant enzyme. HPAEC-PAD on a Dionex ICS3000 system and Mass Spectrometry were used in the reaction studies and product characterization.

Conclusions

A chitinase of 42 kDa from *T. harzianum* was overexpressed in *P. pastoris*, the recombinant protein was purified, characterized and crystallized for the protein structural resolution. Production of COS mediated by this enzyme was evaluated and some of the molecules formed were characterized.