Cloning of the *XynA* gene from *Thermomyces lanuginosus* and expression in *Saccharomyces cerevisiae*

by

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SUMMARY

The aim of the study was to construct an immobilized cell surface expression cassette for the xylanase (*XynA*) from *Thermomyces lanuginosus*. This cassette was constructed by deletion of the binding domain coding region of an α-agglutinin (*Agα1*) gene from *Saccharomyces cerevisiae*, and the cloning of the *XynA* gene from *T. lanuginosus* into the deleted region, which is flanked by a stalk-like protein coding region. The motivation for this was that the enzyme would be expressed on this stalk, on the surface of the yeast cell wall, and that would create an immobilized enzyme system.

Total RNA from *T. lanuginosus* was isolated, and the *XynA* gene amplified with a two-step RT-PCR method using sequence specific primers. This amplified *XynA* was successfully cloned and sequenced. A *PDC1* promoter from *S. cerevisiae* was cloned into two shuttle vectors, pRS416 (single copy) and pRS426 (multi-copy). This modified *Agα1* (with the *XynA* cloned into the deleted binding domain region) was cloned into the two shuttle vectors, adjacent to the *PDC1* promoter, and transformed to *S. cerevisiae*. Upon expression, it was clear that the enzyme was not immobilized, as no enzyme activity was detected when whole-cell enzyme assays were performed. However, activity was found in the extracellular fraction, which indicates that the enzyme was extracellularly secreted. Enzyme assays were thus performed on the extracellular fraction as crude enzyme extract, to complete the partial characterization of the secreted xylanase.

Upon characterization, it was found that the optimum temperature of 70 °C and optimum pH of 6.0-7.0 corresponds to values published in literature. However, the total activity displayed by the recombinant xylanase was significant lower than that of published data. The thermal stability of the recombinant xylanase was also not comparable to that reported. However, it did seem as if the *Agα1*-construct yielded a certain form of stability to the expressed xylanase, as no or very little activity and no stability were found when the xylanase expressed in *S. cerevisiae* without the construct was subjected to enzyme assays.

Further investigation into the reasons why low activity and stability were obtained and no immobilization of the enzyme upon expression by the cassettes could prove valuable in the search for an immobilized cell surface expression system in *S. cerevisiae*.

OPSOMMING

Die doel van die studie was die konstruksie van 'n geïmobiliseerde sel oppervlak uitdrukkingskasset vir die xilanase (*XynA*) van *Thermomyces lanuginosus*. Hierdie kasset is gemaak deur die verwydering van die bindingsdomein koderings gebied van 'n α-agglutinasie geen (*Agα1*) van *Saccharomyces cerevisiae*, en die klonering van die *XynA* geen in hierdie gebied, wat aangrensend is aan 'n steelagtige proteien koderingsgebied. Die motivering hiervoor was dat die ensiem op hierdie steel uitgedruk kan word, op die oppervlak van die gis-selwand, and so 'n geïmobiliseerde ensiemsisteem kan skep.

Totale RNS van *T. lanuginosus* is geïsoleer, en die *XynA* geen geamplifiseer met 'n twee-stap omgekeerde transkripsie polimerase ketting reaksie met spesifieke inleiers. Hierdie geamplifiseerde *XynA* is suksesvol gekloneer en basispaar opeenvolging orde bepaal. 'n *PDC1* promotor van *S. cerevisiae* is kloneer in twee oordragingsvektore, pRS416 (enkel kopie) en pRS426 (multikopie). Hierdie gemodifiseerde *Agα1* (met die *XynA* gekloon in the verwyderde bindingsgebied), is gekloneer in die twee oordragingsvektore, langs die *PDC1* promoter, en getransformeer na *S. cerevisiae*. Na uitdrukking was dit duidelik dat die ensiem nie geïmobiliseer is nie, want geen aktiwiteit is verkry nadat ensiem aktiwiteitsbepalings op die heel selle uitgevoer is nie. Aktiwiteit is wel gekry in die ekstrasellulêre fraksie, wat wys dat die ensiem ekstrasellulêr uitgeskei is. Verdere ensiem aktiwiteitsbepalings is uitgevoer op die ekstrasellulêre fraksie as ru-ensiem ekstrak om die gedeeltelike karakterisering van die uitgeskeide ensiem te voltooi.

Die optimum temperatuur van die uitgedrukte ensiem is bepaal as 70 °C, en optimum pH was 6.0-7.0. Dit is gelykstaande aan waardes wat in literatuur gebubliseer is. Die totale aktiwiteit van die rekombinanate xilanase is egter laer as die van gepubliseerde data. Die temperatuur stabiliteit van die rekombinante xilanase is ook nie vergelykbaar met gepubliseerde data nie. Dit wil egter voorkom of the *Agα1*-konstruk 'n mate van stabiliteit aan die uitgedrukte ensiem verskaf, want min of geen aktiwiteit is gevind met die xilanase uitgedruk in *S. cerevisiae* sonder die konstruk nie.

Verdere ondersoeke na die redes waarom min aktiwiteit en stabiliteit verkry is en geen immobilisering met uitdrukking van die ensiem in die uitdrukkingskasette nie, kan waardevol wees in die soeke na 'n geïmobiliseerde seloppervlak uitdrukkingssisteem in *S. cerevisiae*.