OPTIMISING NATURE FOR INDUSTRY: DESIGN OF SYNTHETIC PROMOTERS FOR STRAIN ENGINEERING OF *TRICHODERMA REESEI*

Elisabeth Fitz[a], Robert Bischof[a], Bernhard Seiboth[a,b]

[a]acib GmbH, Graz, Austria, c/o E166-5 Institute of Chemical, Environmental & Biological Engineering, TU Wien
[b]Molecular Biotechnology Group, Institute of Chemical, Environmental & Biological Engineering, TU Wien, Gumpendorferstrasse 1A, 1060 Wien, Austria

INTRODUCTION

Over the recent years a lot of efforts and research have been made to develop the fuel-based chemical industry towards a bio-based industry. A common approach is the production of biochemicals and enzymes via microbial production hosts.

Trichoderma reesei is a filamentous fungus that is well established for the production of biorefinery enzymes[1,2], mostly cellulases and hemicellulases. Due to its capability to produce high amounts of proteins that are secreted directly into the medium it is also used increasingly to express heterologous proteins. To optimise their production, different genetic tool are necessary to obtain high yields. One of the limiting factors is the transcription levels of the heterologous target genes[3]. Therefore, we develop synthetic promoters with a high basal expression level.

EXPERIMENTS / FUNDAMENTAL OF THE PROBLEM / EXAMINATIONS

The promoter of the uncharacterized gene *cdna1* (P*cdna1*) is one of the strongest promoters that is active during growth on glucose[4]. Although even stronger promoters exist, they are often repressed on glucose or require activation by specific inducers. Our goal was to rationally design a promoter, which is as strong as an inducible promoter but active during growth on glucose.

The project was divided into two parts and focuses on the design of a 200 bp long **core promoter** and an about 1000 bp long **proximal promoter**, which can be combined to a full length promoter. Using RNAseq data from glucose-grown cultures, we analysed 50 highly expressed genes towards their core promoter structure[5]. As references we used the core promoters from *cdna1* and from a strongly expressed phosphatase. Based on the findings we established a box structure system: 6-20 bp long sequences (boxes) were arranged on a 200 bp long either completely artificial or modified natural backbone.

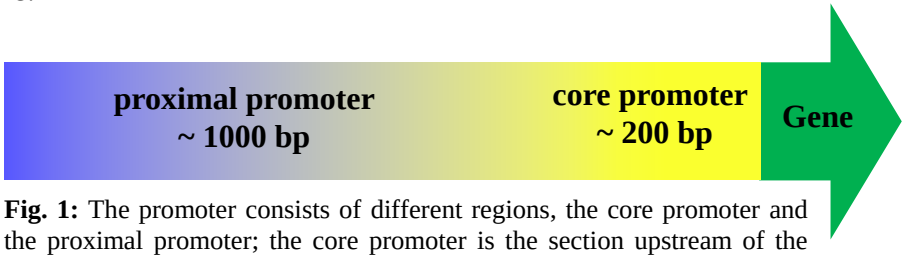

Fig. 1: The promoter consists of different regions, the core promoter and the proximal promoter; the core promoter is the section upstream of the start ATG

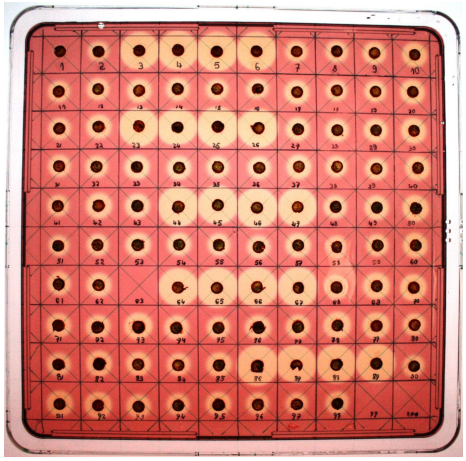

Fig. 2: Cellulose containing agar plate with strains expressing CEL12A under the control of the different synthetic core promoters, stained with a Congo Red solution; CEL12A degrades the cellulose around the gel plug and therefore the dye cannot bind anymore and forms the “clearing zones”;

RESULTS AND DISCUSSION

Six novel core promoters were generated and fused to the endoglucanase CEL12A. The expression strength of the core promoters was tested via a colorimetric plate assay in *T. reesei*: the strains were grown on a cellulose containing agar medium. The agar was then dyed with a Congo Red solution, which led to a discoloured “clearing zone” were the fungi secreted a lot of the cellulase CEL12A. The amount of secreted cellulase is proportional to the expression.

We found that two of the new synthetic core promoters surpassed the expression strengths of the native P*cdna1* and the phosphatase by 175% and 20% respectively. Both of these synthetic core promoters were based on the artificial backbone, whereas the inserted boxes where arranged as in a natural core promoter. The activity occurred solely from the used boxes, including already known sequences like TATA-boxes, GC-boxes or CT-rich regions, but also newly found boxes from our sequence analysis.

CONCLUSION

This work demonstrates that the ration engineering of a core promoter using the box structure can be a tool to further improve the expression strength of already strong core promoters. The next step is the development of a synthetic proximal promoter that further enhances the strength of a synthetic promoter.

This system offers the possibility to combine different characteristics to get the optimised synthetic promoter for different applications.

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