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Haute Ecole Spécialisée de Suisse occidentale
Fachhochschule Westschweiz
University of Applied Sciences and Arts Western Switzerland

Master of Science HES-SO in Life Sciences

Optimization of Lonza's *Pichia pastoris* expression system for the production of therapeutic proteins

Sebastian Scheibler

APPLIED BIOSCIENCES

HES-SO Valais

Advisor: Dr. Sergio Schmid // In collaboration with **Lonza AG, Visp**

DESCRIPTION

The influence of the 5 helper factors HF1, HF2, HF3, HF4 and HF5 on the production of the customer product ABX was tested. In addition, different helper factor/promoter combinations were created to test whether the effectiveness of the helper factor can be increased by another promoter. For this purpose, the constitutive GAP promoter was replaced by the glucose-induced promoters G1 and G6; the procedure is illustrated using the G1 promoter as an example in Figure 1. In addition, two different *P. pastoris* host backgrounds were tested, using strains LS2.3 and LS3 created by Lonza, a double knockout and a single knockout variant of *P. pastoris*.

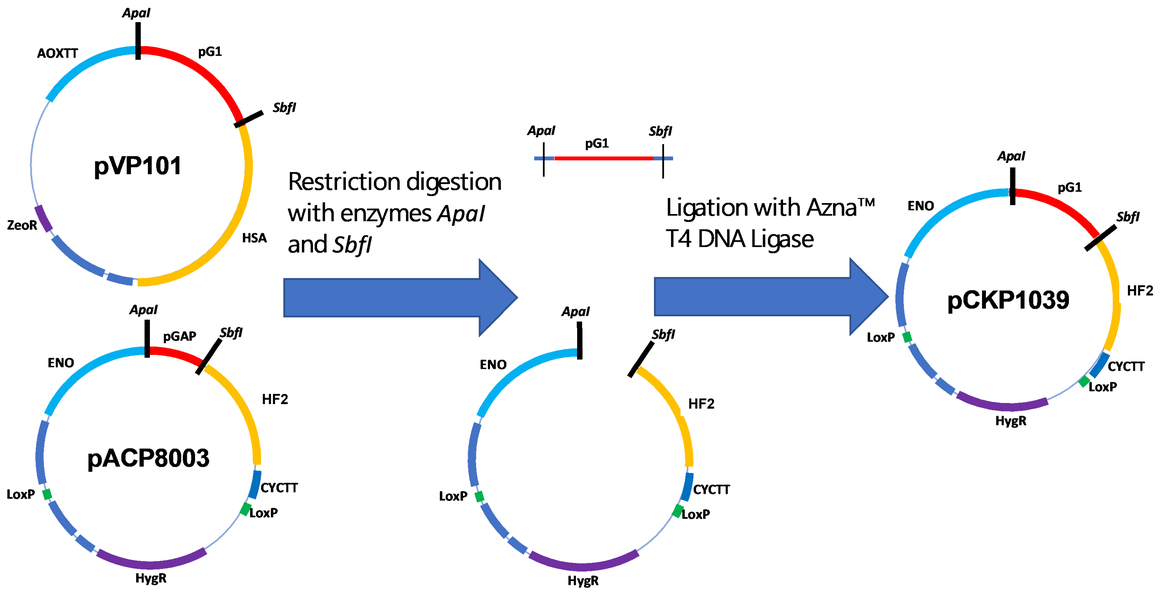

Figure 1: Cloning strategy to replace the constitutive GAP promoter by the glucose-regulated G1 promoter inside the HF2 helper factor plasmid.

The helper factor plasmids were each co-transformed with the plasmid containing the client product into the two *P. pastoris* strains via electroporation. Furthermore, only the customer plasmid was transformed as a reference without any helper factors. Selection was performed by antibiotic resistance, with double resistance (Hygromycin and Zeocin) in the strains with helper factors and single resistance (Zeocin) in the reference. In each case 20 clones were picked and a 24-well screening was performed. The supernatants were analyzed by ELISA and SDS-PAGE.

OBJECTIFS

- Selection of helper factors and helper plasmids (with helper factor genes).
- For the different helper plasmids replacement of the constitutive pGAP promoter regulating the helper factor gene expression with glucose-regulated promoters to enable the glucose-induced co-expression of the helper factor genes.
- Co-transformation of different *P.pastoris* host backgrounds with expression plasmid (one target gene) and different helper plasmids, followed by clone selection.
- 24-well screening to analyze and quantify the target protein production for the isolated clones, data collection and summary

RESULTS

Figure 2 shows a boxplot of the ELISA measurements of the helper factor HF2, which showed the greatest influence on the product titer of ABX. On the left side the data of strain LS2.3 are shown and on the right side of LS3. Within the strains, the reference without helper factor is on the far left, followed by the data for the G1 promoter, then the G6 promoter and finally the GAP promoter.

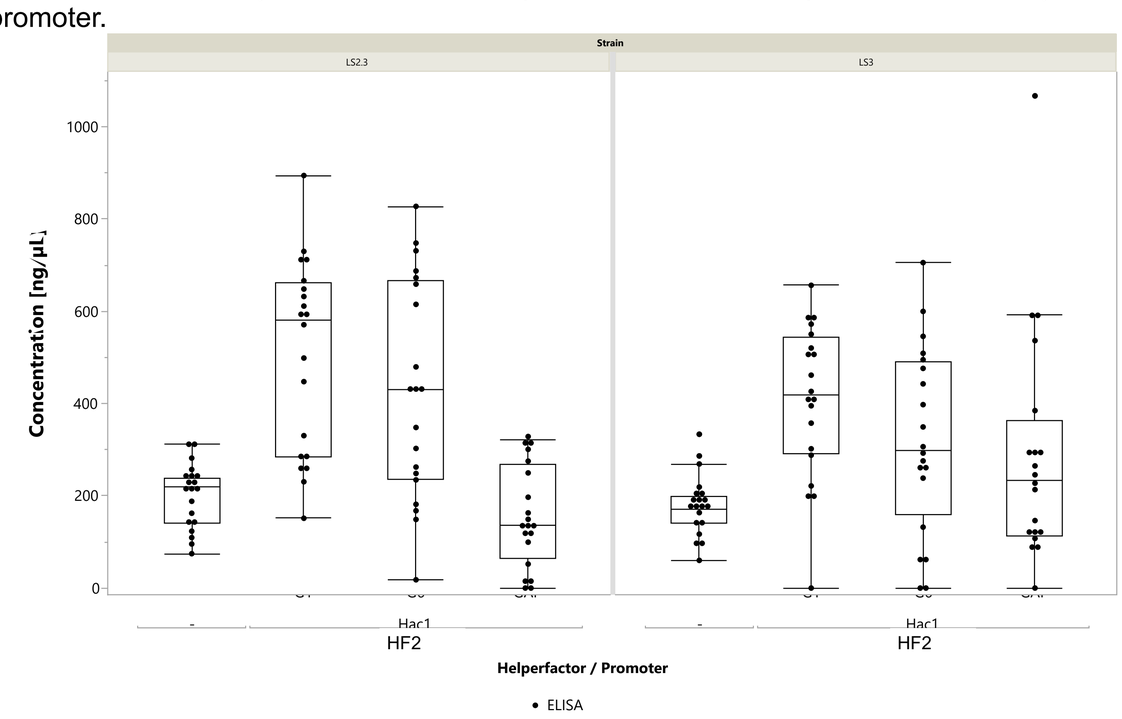

Figure 2: Boxplot of ELISA data of the helper factor HF2

From each helper factor/promoter combination, as well as the reference, the 4 best producing clones were selected and additionally applied to a SDS gel. This required deglycosylation using the enzyme PNGase F with a molecular weight of 36 kDa. Using the SDS gel, it was possible to determine whether the correct product was produced, which has a molecular weight of 38.4 kDa. Figure 3 shows the SDS gel compared to the ELISA data, lane 1 contains the ladder and lane 2 contains the pure product ABX diluted in PBS with a concentration of 310 ng/µL.

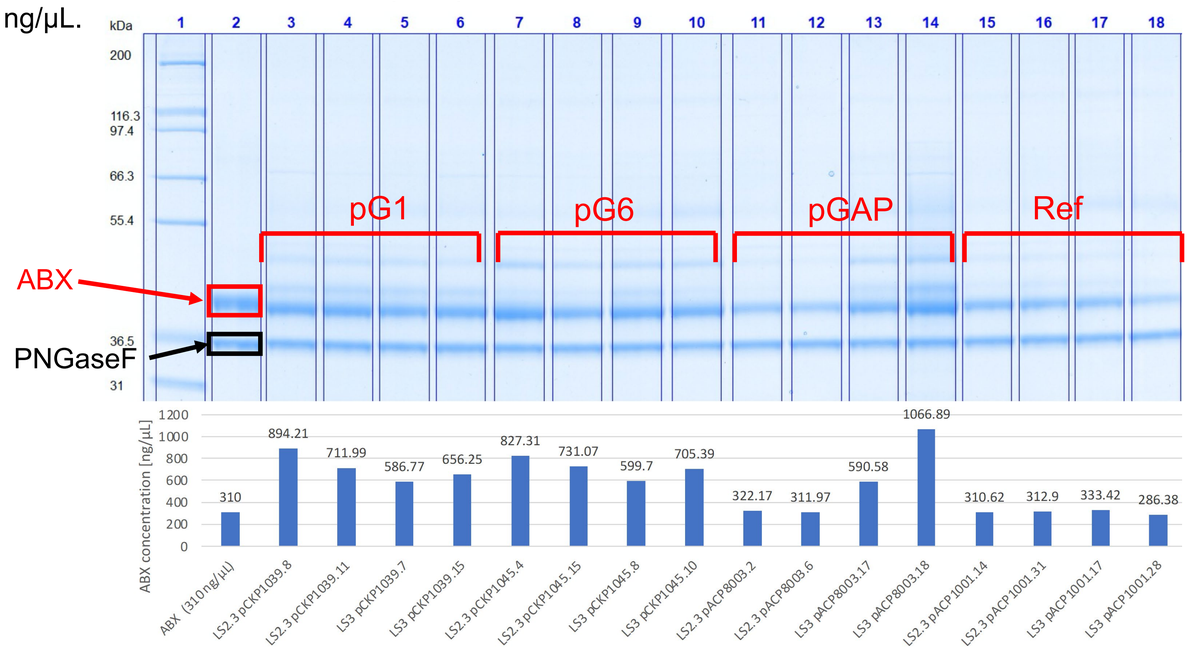

Figure 3: SDS-PAGE gel compared to ELISA data of HF2

CONCLUSION

The replacement of the constitutive GAP promoter by glucose regulated promoters, for which the strong G1 promoter and the weaker G6 promoter were chosen, could be performed for all helper factors. The promoter had an effect on the efficacy of the helper factors, but this varied from helper factor to helper factor, for example, promoter G1 resulted in the highest product titers for helper factor HF2 while promoter G6 did so for HF3. The two tested strain backgrounds, the Lonza proprietary knock-out strains *P. pastoris* LS2.3 and *P. pastoris* LS3, also showed an effect on the product titer of ABX, strain LS2.3 resulted in higher productivity for all helper factor/promoter combinations. Of the helper factors themselves, only HF2 showed a significant effect on the concentration of ABX, up to 2.5 times higher product titers could be achieved. The helper factor HF3 led to a slightly increased product titer, while HF1 and HF4 showed no influence. HF5 was the only helper factor that led to reduced productivity, only 80% of the ABX concentration could be achieved compared to the reference. In summary, two factors influence the effectiveness of helper factors, namely the type of product and the promoter that regulates the helper factor. In addition, the strain background also has an influence on productivity. Further steps in this work would be, testing combinations of helper factors, as well as investigating the copy number and finally to confirm the results a secondary screening in form of a fed-batch culture.

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