

Production of heterologous proteins by different microbial organisms using novel expression systems



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Introduction

For industrial applications and (medical) scientific research efficient (heterologous) production of compounds like, enzymes, antibodies, viral epitopes, chemical compounds and antibiotics is of great importance. Over the years all kinds of commercially available expression systems have been developed. However, in the case of heterologous protein production the efficiency of these systems is still very depending on the protein to be produced. For our contract research projects at the HAN BioCentre we have successfully applied several expression systems and different host organisms like *Escherichia coli*, *Lactobacilli sp.* and the filamentous fungus *Aspergillus*. Recently, we have developed and protected a new efficient and controllable expression system for *Aspergillus*. Based on the combination of our expertise on construction of efficient production hosts and our experience with their cultivation in our state-of-the-art fermentation facilities we offer all expertise to achieve optimal production of heterologous proteins.

Expression systems

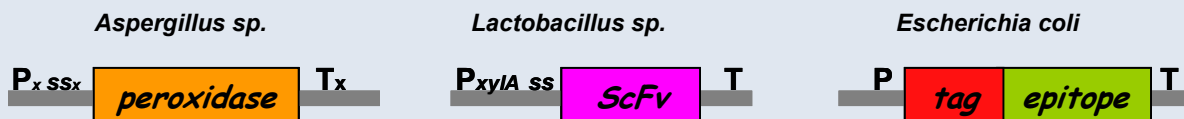


Figure 1a: Expression cassette *Aspergillus* expression vector.

Px: inducible promoter. ss: signal-sequence. Tx: termination sequence.

Figure 2a: Expression cassette *Lactobacillus* expression vector.

PxyIA: inducible promoter. ss: signalsequence usp45.

Figure 3a: Expression cassette *E. coli* expression vector. Proteins are isolated and purified using tags like His, GST, and MBP.



Figure 1b: O-anisidine plate assay of peroxidase producing *A. niger* strains.

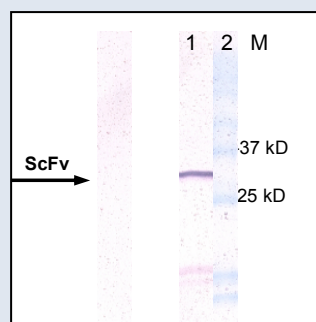


Figure 2b: Western blot analysis of ScFv producing *L. casei* strain. 1: *L. casei* wt; 2: ScFv producing *L. casei* strain; M: marker.

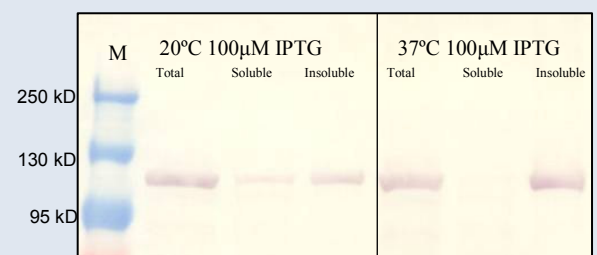


Figure 3b: Western blot analysis of a virus epitope producing *E. coli* strain under inducing conditions (IPTG) at different temperatures.

Conclusion / Future Plans

At HAN BioCentre a variety of heterologous proteins are produced successfully in different host organisms. With our novel *Aspergillus* expression system we were able to produce a peroxidase. ScFv could be successfully produced in *L. casei* and different virus epitopes were produced efficiently using *E. coli* as host organism. Cultivation of *E. coli* producing strains at 20°C in stead of 37°C resulted in an increase of soluble protein which could be isolated easily and efficiently using His-, GST- and MBP-tags, respectively. Adjustment of cultivation conditions during (fed-)batch fermentations resulted in improved production levels. Future research will focus on optimization of different expression systems.