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Development of a *P. pastoris* strain for the recombinant production of peptide-based antibiotics in eukaryotic cells

Introduction

In consequence of rising bacterial resistance to antibiotics, new effective antibiotics are needed. One alternative to conventional antibiotics are **antimicrobial peptides (AMPs)**. AMPs are small peptides which own various **mechanisms against bacteria, fungi, protozoa and viruses**.

The amphiphilic secondary structure allows the in total positively charged AMPs to interact with the negatively charged microbial cell membrane and permeabilize this. The integrity of the microbial cell is destroyed. This particular mechanism acts **specifically against microbes**, without influencing adjacent animal cells.[1,2]

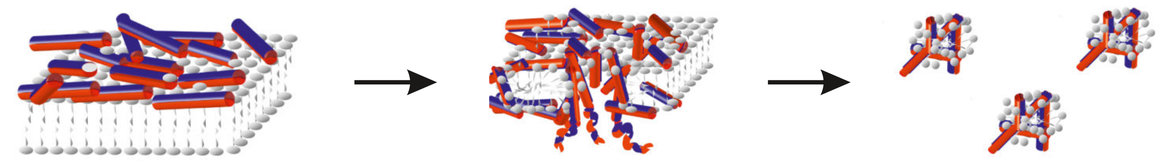


Fig. 1: The Carpet model, one possibility to explain the interaction of the AMPs with the bacterial cell wall. (Adpated from Shai & Oren (2001) Peptides).

The objective of this work is the production and purification of recombinant AMPs using a *P. pastoris* strain, which first secrets an AMP-fusion protein to overcome the autolytic effect on the eukaryotic host organism.

1. Strain development

For recombinant AMP production, a *P. pastoris* strain, expressing an inactive fusion construct, is designed. GFP fusion is likely to improve cellular production. Moreover, it enables on-line process analytics (PAT) via in-line fluorescence detection.

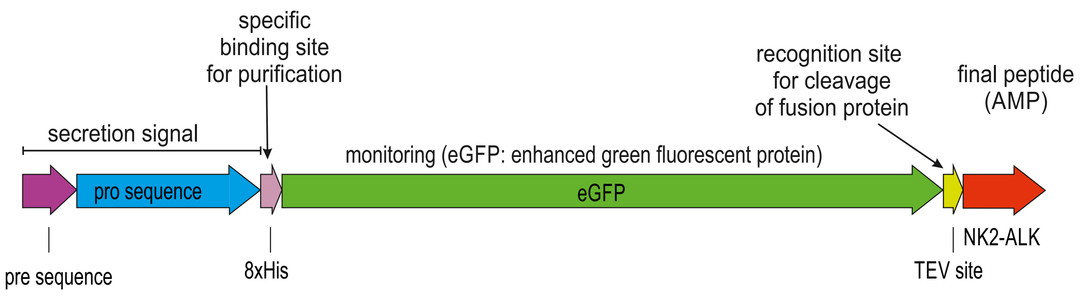
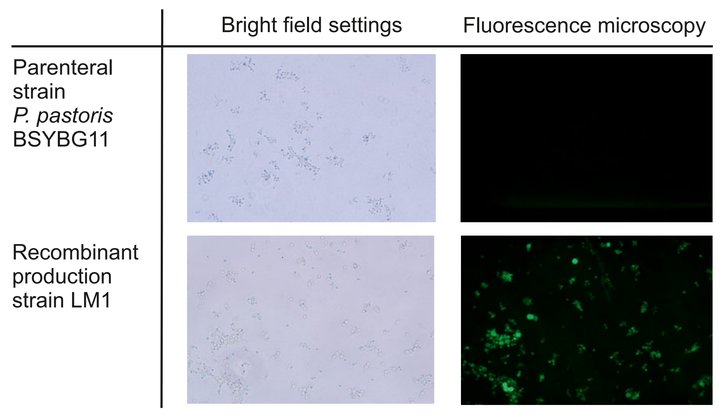


Fig. 1: Schematic representation of the fusion construct.

As proof of principle, the clones were applied in small-scale expression experiments.

Fig. 2: Images of the same field of view were taken. Whereas the parental strain shows no fluorescence signal 48 h after induction, at the recombinant production strain a fluorescence signal is detected.



2. Cultivation

Expression experiments were performed in a highly automated 5 l lab-scale bioreactor. A fully automated 3-stage upstream process was carried out. During batch phase *P. pastoris* is grown on glycerol, followed by a controlled exponential fed-batch phase. An in-line methanol measurement control circuit controls gene expression during production phase.

After methanol induction the expression of the fusion protein was successfully monitored by an in-line fluorescence probe.

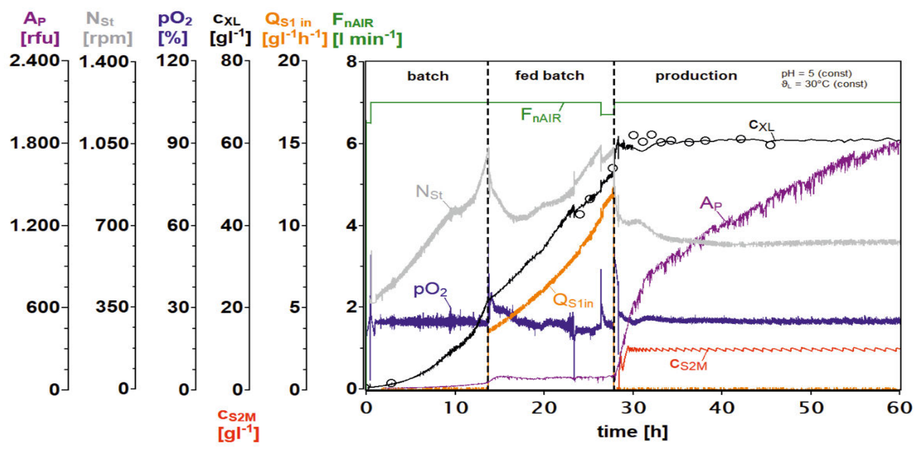


Fig. 3: Cultivation plot: Time courses of the on-line fluorescence signal (A_P), the agitation speed (N_{St}), the dissolved oxygen tension (pO_2), the cell density (c_{XL}), the methanol concentration (c_{S2M}), the volumetric glycerol uptake rate (Q_{S1in}) and the flowrate of air (F_{nAIR}).

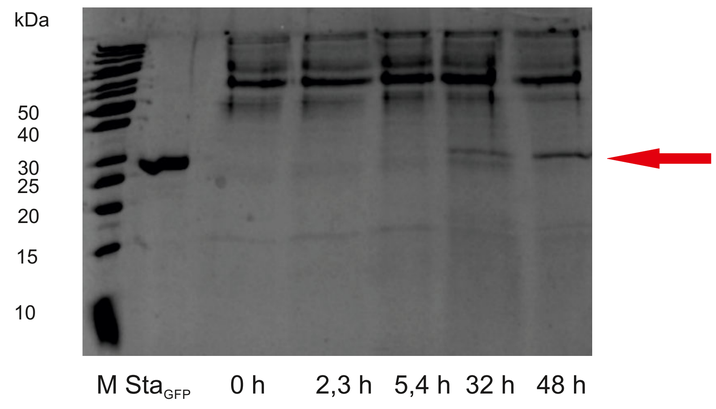


Fig. 4: The cultivation supernatant was analysed by SDS-PAGE. Bands with the mass of the fusion protein (32 kDa) were observed 32 h after methanol induction. M, protein ladder; Sta$_{GFP}$, GFP standard.

3. Purification

Past sterile filtration, the collected supernatant was applied to a 1 ml HisTrap™ Fast Flow Crude column (GE Healthcare).

As flow rate 1 ml min^{-1} was chosen. First, the column is equlibrated with buffer A and the product solution is loaded onto the column. Stepped elution was performed by changing to 100 % of buffer B.

The observed peak was fractioned and pooled followed by analysis via SDS-PAGE.

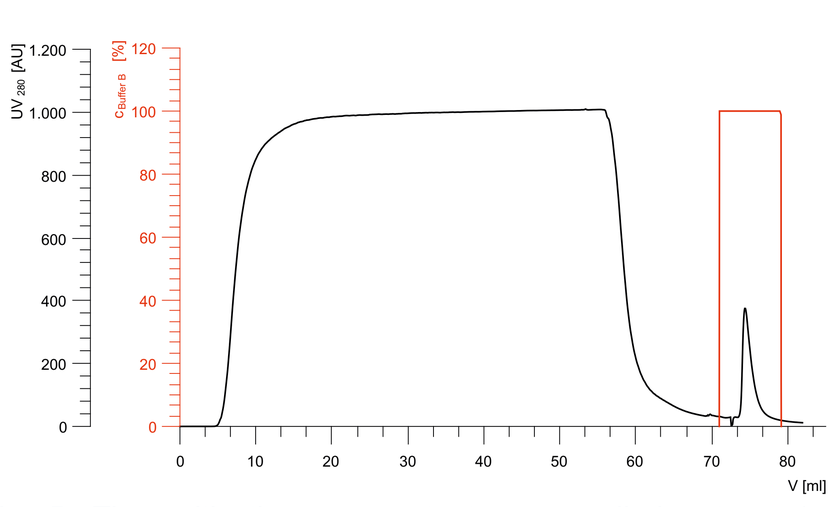


Fig. 5: The cultivation supernatant was applied onto a 1 ml HisTrap™ column. After elution by changing to 100 % of buffer B the peak fractionation was started, allowing further analysis steps.

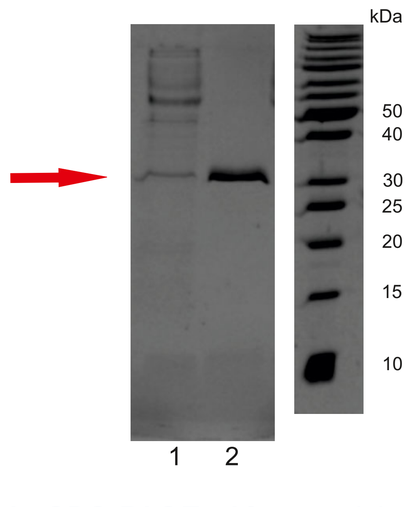


Fig. 6: SDS-PAGE: After applying the cultivation supernatant (lane 1) onto the HisTrap™ column, exclusively one band with the expected mass of the fusion protein was observed (lane 2).

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References

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[2]Zasloff, M.: Antimicrobial peptides of multicellular organism. Nature, Bd. 415, Nr. 0028-0836 (Print), S. 389-395, 2002.

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