Master's Thesis Engineering Technology

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Biotechnological Production of Nanobodies Against Ovarian Cancer

Anneleen Coun

Master of Biochemical Engineering Technology

Figure 1: The variable fragments of antibodies derived from camelids are called nanobodies [1]

INTRODUCTION

Camelids have unique antibodies in their blood in contrast to other mammalian species. The variable fragments of these antibodies are called nanobodies (Nbs). Due to their small size and high stability, nanobodies are an ideal tool to be used in detection methods for cancer. Epithelial ovarian cancer for example, is a metabolic disease, which affects postmenopausal woman. It is desired to detect this cancer at an early stage to increase survival rate and reduce side effects of chemotherapy.

OBJECTIVES

This thesis optimizes the biotechnological production of nanobodies processed as a fusion protein in three *Escherichia coli* strains to be used in detection methods for epithelial ovarian cancer. One part is the optimization of the expression parameters. Another part is the purification optimization on a chitin column, in order to obtain the nanobody itself.

METHODS

Growth

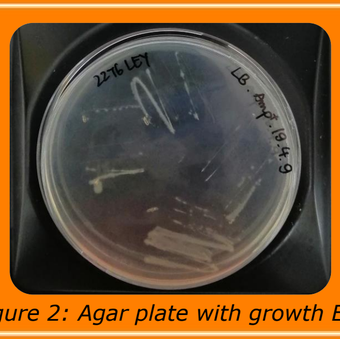

Figure 2: Agar plate with growth E. coli

Growing of *E. coli* (with the nanobody gene) on a plate with growth medium and ampicillin to avoid the growth of other bacteria

Expression fusion protein

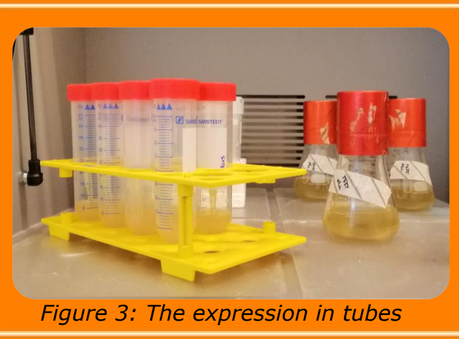

Figure 3: The expression in tubes

Varied parameters:
- **Temperature**
- **Medium**
- **Time**
- **Concentration inducer**

Nanobody purification

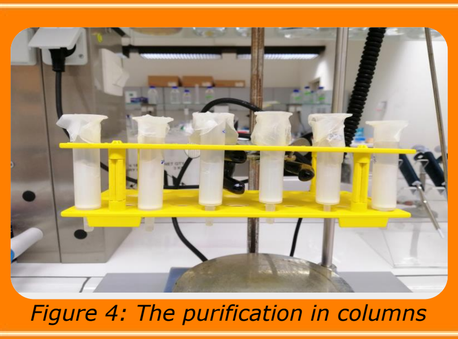

Figure 4: The purification in columns

Varied parameters:
- **Cleavage time**
- **Cleavage temperature**

Evaluating results

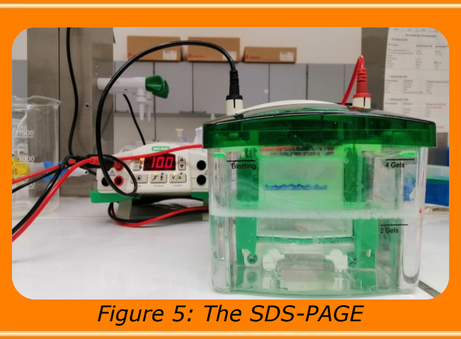

Figure 5: The SDS-PAGE

- **SDS-PAGE: evaluating expression**
- **Nanodrop: concentration measurement of the nanobodies**

RESULTS

At first, different values of the expression parameters were tested to determine the optimal settings for a high expression of the fusion protein (~42kDa). These optimal settings are identified based on several SDS-PAGE gels and are given in Table 1. The SDS-PAGE result of the optimal expression experiment is given in Figure 6.

- Strain 2210: high intensity band between 35 and 55 kDa for both media.
- Strain 3237: lower intensity band between 35 and 55 kDa, in contrast to strain 2210.
- Strain 2276, no conclusions can be made since the intensity of the band between 35 and 55 kDa is too weak.

Table 1: The optimal parameter settings for protein expression

Temperature	37 °C
Medium	Luria Bertani (LB) Terrific Broth (TB)
Concentration	0.1 mM
Time	2 – 3 hours

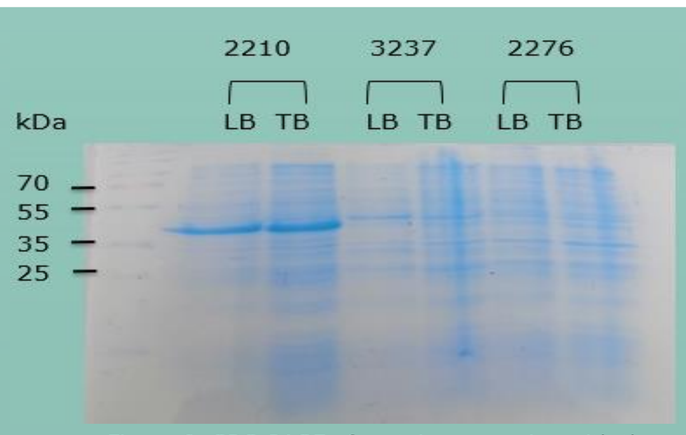

Figure 6: SDS-PAGE of protein expression with the optimal parameter settings

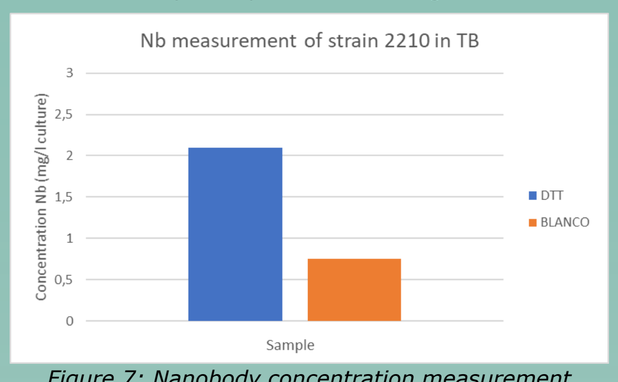

Figure 7: Nanobody concentration measurement through Nanodrop

Next, the purification of the expressed protein via affinity chromatography based on a chitin column was studied in terms of cleavage temperature and time. The purification was done with the expressed proteins following the optimal expression parameter settings as given in Table 1, in TB medium for 2,5 hours.

- No clear results for the optimal purification parameter settings were obtained.

Nevertheless, the concentration of the nanobody from each strain was measured.

- The highest nanobody concentration was found in strain 2210 and is represented in Figure 7.
- However, the measured concentration is low and therefore, no reliable conclusions can be made.

CONCLUSION

The optimization of the expression parameters was successfully completed and reference is made to Table 1. For the optimal purification parameter settings, future research is still needed since the results obtained from SDS-PAGE and Nanodrop are not reliable. More accurate concentration measurements can be performed by the 'Bradford protein assay'.

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Dr. Graulus
Ing. Baillien

[1] Thinkstock, „Het leukste van het web," 11 December 2017. [Online]. Available: https://www.hln.be/bizar/het-leukste-van-het-web/los-jij-onze-maandagpuzzel-op-het-testament-met-17-kamelen~a13e053b/. [Geopend 2 Mei 2019].

[2] D. T. Ta *et al.*, "An efficient protocol towards site-specifically clickable nanobodies in high yield: Cytoplasmic expression in Escherichia coli combined with intein-mediated protein ligation," *Protein Eng. Des. Sel.*, vol. 28, no. 10, pp. 351–363, 2015.

UHASSELT

KU LEUVEN