OPTIMIZATION OF A BACTERIAL SECONDARY DEHYDROGENASE (sADH) EXPRESSED IN *ESCHERICHIA COLI* BL21 DE3

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Introduction. Proteins used for research or industrial purpose are mainly produced in microorganisms. Due to its well-known genetics as well as its inexpensive cultivation, *E. coli* is a commonly used host for the production of recombinant protein. The main obstacle for a large scale production of protein in bacterial systems is the accumulation of misfolded, insoluble and thus nonfunctional protein bodies during synthesis. Those are known as inclusion bodies. There are different methods to overcome that obstacle, either by in vitro refolding or by modifying the cultivation conditions. The protein of interest is the secondary alcohol dehydrogenase (sADH) from *Rhodococcus erythropolis* DSM 44534. The enzyme possesses activity towards γ-nonalactol regarding the corresponding γ-nonalactone (1), which is an important ingredient for the food industry.
According to previous research, the expressed sADH in *E. coli* via the pET system (2) induce the protein aggregation and in the present work, we describe how to decrease the aggresome formation in *E. coli* by varying the cultivation procedure

Methods. 10 mL LB medium were inoculated with a single colony of transformed *E.coli* BL21 DE3 star containing pET29a/ADH plasmids. The cultures were grown to an OD600 of 0.6-0.8 at 37°C and protein expression was induced by adding different concentrations of isopropyl-β-D- thiogalactoside (IPTG) to a final concentration of 0.25, 0.5 or 1mM and different temperatures of 25°C, 28°C and 37°C. The cultures were induced for 2 hours and harvested subsequently by centrifugation at 4°C, 5000rpm for 10 minutes. After dissolving the cells in10mM Tris-HCL buffer added with 1% n-butanol, cells were disrupted using a Potter Homogenisator. SDS-PAGE was performed to evaluate the protein aggregation

Results.
After cell disruption, the supernatant and the pellet fraction were evaluated via SDS page and stained using Coomassie Blue. Figure 1 displays the samples that were induced with either 1mM or 0,5 mM IPTG, following cultivation at 25°C (fraction 1-4). Figure 2 shows samples that were inducted with 0,5mM/1mM IPTG and afterwards cultivated at 28°C (fraction 5-9). The protein of interest has a size of 55kDa.

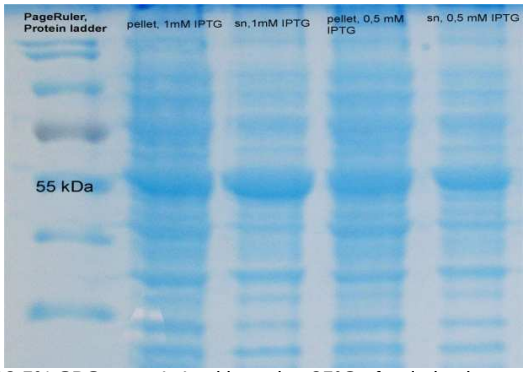

Fig. 1: 12,5% SDS page 1-4 cultivated at 25°C after induction

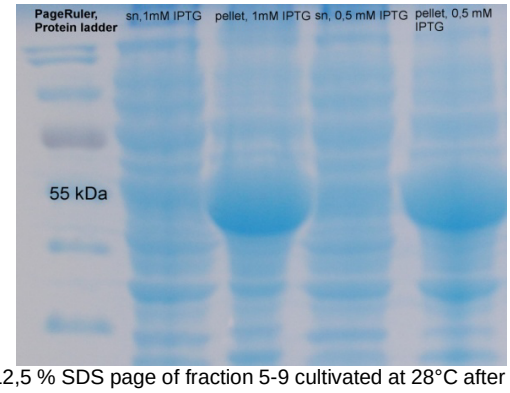

Fig. 2: 12,5 % SDS page of fraction 5-9 cultivated at 28°C after induction

Conclusions.
The formation of inclusion bodies could be reduced by decreasing the cultivation temperature after induction via IPTG to 25°C and performing cell disruption in 10mM Tris-HCL buffer containing 1% n-butanol. The obtained soluble protein can be used for further research.

References.
1.- Martinez-Rojas, E, and Garbe, L.-A. (2012) A novel secondary alcohol dehydrogenase from *Rhodococcus erythropolis*. Catalyzing Bio-Economy - Biocatalysts for Industrial Biotechnology, Annual meeting of the DECHEMA-VAAM-Section Biotransformations.. Frankfurt, Germany.April 24th-25th. Pag. 29
2.- Kurt, T. C., Martinez-Rojas, E., Schmidt, U., Meyer, V. and Garbe, L.A.(2012). Cloning and heterologous expression of a gene encoding NAD-dependent (S)- alcohol dehydrogenase from *Rhodococcus erythropolis* DMS 44534. Sustainable industrial biocatalysis. International symposium of sustantable bioeconomy. BiochemGate GmbH. Dortmund, Germany, Nov. 29th to 30th. Pag. 14