**Temperature Effect in the Production of a Recombinant Antivemon in Fed-Batch Mode.**

Susana Alonso Villela1, Hazar Kraiem2, Balkiss Bouhaouala2, Carine Bideaux1, Cesar Arturo Aceves Lara1, Luc Fillaudeau1

*1 LISBP, Université de Toulouse, INSA, INRA, CNRS, Toulouse, France; 2 Laboratoire des Venins et Molécules Thérapeutiques, Institut Pasteur de Tunis, 13 Place Pasteur, BP-74, 1002 Le Belvédère, Tunis, Université Tunis El Manar, Tunisia.*

*\*Corresponding author: alonsovi@insa-toulouse.fr*

**Highlights**

* Production kinetics was obtained during expression at 28, 30, 33 and 37°C
* Lower expression temperatures yielded higher protein than higher temperatures
* The highest productivity was 0.046 mg/g cdw/h, attained after 10 h at 30°C

**1. Introduction**

The production of recombinant antibodies in *E. coli* has been widely studied in shake flasks [1], mainly for the study of protein yields under the *lac* promoter expression system. The effect of the inducer (IPTG) concentration during induction has been studied [2-3], but there are no studies on the temperature effect on protein production kinetics. The duo temperature-duration after induction most commonly used is either 37°C-4h or 28°C-12h and only final titers are reported.

In this work the effects of temperature in the production of the chimeric format of bispecific nanobody NbF12-10 against AahI’/AahII toxins of scorpion venom in *E. coli* WK6 were studied at bioreactor scale. The strategy implemented allowed first the production of biomass in fed-batch mode at 37°C, and then the expression of protein under glucose feed and induction temperatures of 28, 30, 33 and 37°C.

**2. Methods**

Experiments were conducted with *Escherichia coli* K12 / WK6 { ∆(lac-pro), galE, strA, nal; F’ lacIq Z∆M15, pro+ } harboring pHEN6 plasmid (derived from pBR322) encoding the bispecific nanobody VHHF12-VHH10 (called NbF12-10) and the chimeric format VHH10-VHHF12 (called CH10-12) retrieved from the combinatorial libraries. Cultures were performed in a 5L bioreactor, Biostat B-DCU (Sartorius) using glucose as carbon source in 1.5 L of defined medium [4]. Batch phase was carried out at 37°C, and at depletion of the 10 g/L of initial glucose, fed-batch mode was applied with an exponential feed imposing a specific growth rate of µ = 0.38 h-1. Protein expression was induced with 1 mM of IPTG when biomass reached approximately 26 g cdw/L. At induction, glucose feed rate was set to 4.5 g Glc/h (at 300 g Glc/L) imposing a µ ≤ 0.03 h-1. Temperature and duration after induction were set to 28°C-12h, 30°C-10h, 33°C-6h, and 37°C-4h. Cell samples were taken every 2 h for the experiments at 28 and 30°C, and hourly for experiments at 33 and 37°C. After pelleting the cells, the periplasmic proteins were extracted by osmotic shock and purified by IMAC using His-Select Ni affinity gel (Sigma-Aldrich). After washing with PBS, His-tagged proteins were eluted with imidazole. Biomass was measured by optical density and gravimetric method. Residual glucose and organic acids were quantified by HPLC (Aminex column HPX87H). Quantification of protein was made by a new method of image analysis of electrophoresis gels [5].

**3. Results and discussion**

The biomass production phase of the four cultures showed high reproducibility, obtaining a µmax of 0.71 h-1 in batch mode and the expected µ of 0.38 h-1 in exponential feeding fed-batch. During protein expression, a linear growth was maintained, sufficient to satisfy both bacterial and protein production. The protein was produced only after the induction with IPTG. The induction at 37°C was extended and stopped at 6 h. After 5 h of induction, the highest protein yield was 0.015 mg/g cdw at 28 and 30°C, and decreased with the temperature, down to 0.005 mg/g cdw at 37°C. The maximum protein yield achieved was 0.074 mg/g cdw (Figure 1a), after 10 h of induction at 30°C, which is two-fold the yield achieved for the same induction duration at 28°C (0.04 mg/g cdw).

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| a) |  | b) |  |

**Figure 1.** a) Biomass and protein yield evolution in all cultures, and b) Specific production rate of the nanobody produced after IPTG pulse. Symbols: ◊ 28°C, □ 30°C, ∆ 33°C, ○ 37°C.

The specific production rate of the protein (Figure 1b) increases after 5 h of induction for all cultures, obtaining the highest productivity of 0.045 mg/g cdw/h at the induction temperature of 30°C. This is more than five-fold the productivity at 28°C at the same production time (10 h), and two-fold the productivity at 28°C at 12 h.

**4. Conclusions**

The effect of temperature during the induction of a periplasmic-expressed nanobody was tested at bioreactor scale. Culture repeatability was demonstrated, and protein production was achieved during glucose feed. The highest protein titers were obtained at the induction temperature of 30°C. The production of the nanobody at 30°C should be analyzed over a longer period of time to determine the production limits of the strain.

*Susana Alonso acknowledges financial doctoral support by the CONACYT (Mexico).*

**References**

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