

POSTER PRESENTATION

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The influence of *Escherichia coli* cultivation temperature on interferon alpha 2a expression (IFN- α 2a)

Fernanda dos Santos Arthuso^{1*}, Miriam Fussae Suzuki¹, Nélio Alessandro de Jesus Oliveira², João Ezequiel de Oliveira¹, Paolo Bartolini¹, Carlos Roberto Jorge Soares¹

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Background

The Hormone Group of IPEN develops researches on recombinant pituitary hormones produced in *Escherichia coli* at laboratory scale, including human growth hormone (hGH) and prolactin (hPRL). The best results were obtained using the λP_L promoter and the signal peptide DsbA, and so the protein of interest is secreted into the bacterial periplasmic space, in its authentic form without an initial methionine [1,2]. The expression, under control of the λP_L promoter, regulated by the thermosensitive repressor, is used for large scale production in *E. coli*. Thermal induction presents a great advantage over chemical inducers like IPTG or nalidixic acid, that are expensive and dangerous for manipulators and environment, but it presents the disadvantage that proteins particularly thermolabile like hPRL could suffer proteolysis and aggregation, influencing negatively the production. Considering the limitations on the use of IPTG and the periplasmic secretion, successfully obtained with our hGH expression vector using λP_L promoter and W3110 strain, we decided to use this system for IFN- α 2a production. The goal of this work was the construction of the IFN- α 2a vector, obtaining an the IFN- α 2a periplasmic expression.

Methods

The DNA sequences corresponding to the NdeI restriction site, start codon, signal peptide DsbA, cDNA of the IFN- α 2a, stop codon, and the restriction site BamHI were inserted into the plasmid, λP_L -DsbA-mPRL [3]. This plasmid contains the λP_L promoter and the gene

for ampicillin resistance [1]. The new vector was called λP_L -DsbA-IFN α 2a. The IFN- α 2a sequence was synthesized by the GenScript Corporation (Piscataway - NJ - EUA) in the pUC57 plasmid. The vector obtained was amplified in *E. coli* DH5 α strain and then introduced into the expression W3110 cells. After selection, the best clone was used to test different cultivation temperatures (32°C, 35°C, 37°C, and 42°C). Analyses of the periplasmic fluid obtained by osmotic shock based on Western Blotting and SDS-PAGE were carried out.

Results and conclusions

Construction of λP_L -DsbA-IFN α 2a plasmid was confirmed by restriction analysis. IFN- α 2a secreted into the periplasmic space of *E. coli* was obtained, as shown in Western Blotting and SDS-PAGE. We can observe that the best growth temperature for the IFN- α 2a was 37°C probably because at this temperature the bacteria presented a better growth and the production of aggregates was lower. The production yield was of $\sim 0,1 \mu\text{g/ml/A}_{600}$.

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Authors' details

¹Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP, São Paulo, Brazil. ²Lillehei Heart Institute - University of Minnesota, Minneapolis, MN 55455, USA.

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¹Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP, São Paulo, Brazil

Full list of author information is available at the end of the article

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