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

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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 λ P_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 λ P_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 λ P_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 λ PL-DsbA-mPRL [3]. This plasmid contains the λ P_L promoter and the gene for ampicillin resistance [1]. The new vector was called λ PL-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 λ PL-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 ~ 0,1 μ g/ml/A₆₀₀.

Acknowledgments

This work was supported by FAPESP, São Paulo (Project n. 2007/59540-3) and by CNPq, Brasília (Projects GD 141221/2011-9, PQ 303839/2008-2, PQ 3000473/2009-5 and DT 310512/2010/7).

Presentation type: Abstract for voluntary poster presentation

Session: Other areas of biotechnology

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5th Congress of the Brazilian Society of Biotechnology (SBBIOTEC5)

**Oceania Convention Center – Florianópolis, SC,
Brazil, November 10 - 14, 2013**

Other areas of biotechnology