Amplification and cloning the gene of bovine leukocyte α-A-interferon in cells of *Escherichia Coli*

Arafat A. Muttar*
Department of Missions and Cultural Relations, Ministry of Higher Education and scientific research, Baghdad, Iraq.

Abstract

Interferon's plays a role in innate immune responses through upregulation of costimulatory molecules and induction of proinflammatory cytokines. Interferon alpha (IFN α) type of Interferons. The present study characterized IFNα cDNA . The interferon's play a great role in protection from infections, caused by microorganisms, and have powerful antiproliferative and immunomodulation activity. In this study DNA was isolated from bovine blood leukocyte, which was used in the quality of matrix for amplification of α-interferon gene with the use of PCR, and isolation of gene α-interferon and transformation in vector pUC18 and expression vector pET24b (+). All plasmids contained an additional DNA fragment size corresponding to the gene of bovine α-A-interferon.

Key words: interferon, plasmids, bacterial strains.

تضخيم وكلونة جين الانترفيرون الفا من كريات الدم البيضاء للأبقار في بكتريا القولون *Escherichia Coli*

عرفات عبد الرزاق مطر*
دائرة البعثات والعلاقات الثقافية ، وزارة التعليم العالي والبحث العلمي، بغداد، العراق.

الخلاصة

الانترفيرونات تلعب دورا في الاستجابة المناعية الذاتية من خلال اعادة التنظيم وتحفيز السايتوكاينيز ، ولها نشاط مناعي . الانترفيرون ألفا (IFN α) احد أنواع الانترفيرونات. في هذه الدراسة توصيف الانترفيرن الفا. الانترفيرون تلعب دور كبير في الحمايةمن الاصابات المتسببة بواسطة الاحياء المجهرية ولها نشاط قوي ضد التكاثر والتنظيم المناعي. في هذه الدراسة تم عزل الحمض النووي من كريات الدم البيضاء من الابقار، وتضخيم جين الانترفيرون المعزول من الدنا بمساعدة PCR، ومن ثم التحول في ناقل pUC18 وبعدها التحول البكتيري بواسطة ناقل $pET24b^{(+)}$. حيث أظهرت النتائج وجود جين الانترفيرون الفا البقري في جميع البلازميدات بعد الكلونة.

Introduction:

Interferons are species-specific cytokines, representing a group of biologically active proteins and glycoproteins synthesized by the cells in the immune reaction in response to agents stimulatory [1, 2]. Interferons which are heterogeneous in composition, molecular, weight ranges from 15 to 70 kDa. The system of vertebrates consists of interferon genes and interferon receptors, and effector molecules,

*Email: arafataam3@yahoo.com

related to three types: type I - interferon-alpha (α),-beta (β),-delta (δ),-epsilon (ε), - kappa (κ),-omega (ω),-tau (τ),-zeta (ζ), and others; type II - interferon-gamma (γ) and type III - interferon-lambda (λ) [3]. Genes of these three types of interferon are located on different chromosomes [4, 5].Interferon's are useful in the case of many human diseases: in leukemia, Kaposi's sarcoma, myelogenous leukemia, practically, lymphoma, chronic illness and chronic infection, hepatitis B and C viruses. Interferons are a class of glycoproteins that have antiviral, antiproliferative and immunoregulatory activity [6]. In cows (Bos taurus) found at least 8 genes encoding α-interferon.

The aim of this study: cloning gene bovine α-A-interferon in the bacteria *E. coli* to obtain purified bovine α-A-interferon.

Material and Methods:

The bacteria strain *E. coli* XL-1 Blue (F 'proAB lacIqlacZΔM15 Tn10 (Tcr) / recA1 endA1 gyrA96 (Nalr) thi-1 hsdR17supE44 relA1 lac) supplied from the collection of the Department of Molecular Biology, Faculty of Biology, Belarusian State University was used for cloning recombination plasmids[7]. The cells of *E. coli* BL21 (λDE3) (hsd galλcIts857ind1Sam7nin5lac UV5-T7gen1), lysogenic for bacteriophage λDE3. Plasmid pUC18 used as a vector for the cloning of the gene sequence of cow α- interferon. Plasmid pET24b$^{(+)}$ was used as an expression vector [8]. Total DNA was isolated [9,10], isolation, restriction analysis of recombinant plasmid carrying ca^{+2} dependent transformation and DNA electrophoresis performed with generally accepted experiment protocol. The enzymes and buffer system MBI fermentas (Lithuania) was used. Polymerase chain reaction (PCR) performed in a mixture of standard composition with the use of programmable thermostat: Veriti (96 well thermal cycle).

Primers for PCR designed on the basis information from a database of nucleotide sequences of GeneBank (NCBI). Sequencing performed by the method of Sanger on the sequencer ALF Express.

Results and discussion:

Gene amplification of bovine leukocyte α-interferon was determined applying PCR, the DNA used was isolated from cow blood. Primers designed from Gene Bank nucleotide sequences database (codes M10952, M10953, M10955, M10955): (Table 1)

Table 1- Characteristics of the primers CabF1 и CabR1

Name	Sequence 5'-3 '(restriction enzyme sites are underlined)	restriction enzyme
CabF1	5-GGC CATATG ATG GCC CCA GCC TGG -3	*Nde* I
CabR1	5-gcg GAATTC GAT AAGCTT TCA GTC CTT TCT CCT -3	*Eco* RI, *Hind* III

To optimize PCR amplification various annealing temperatures of the primers (50, 55, 60)°C performed, whereby it was found that the highest yield of the desired product occurs at a temperature of 55°C.

The size of the amplification product of the gene of bovine α-interferon was 498 bp., which fully corresponds to the expected figure-1.

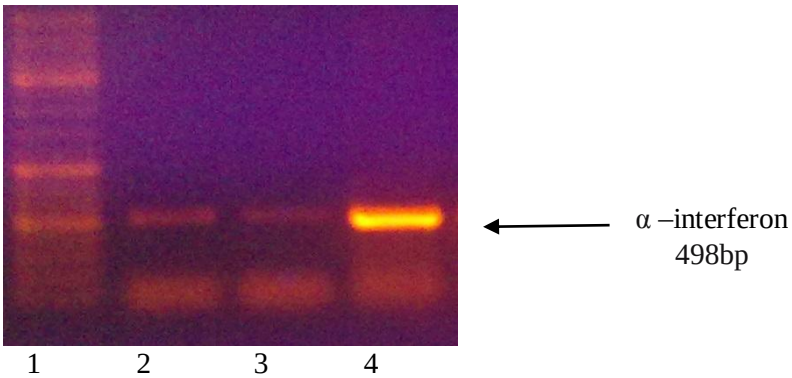

Figure 1 - Gene amplification of bovine leukocyte α-interferon at different annealing temperatures primers. Line 1 - molecular weight marker (Fermentas SM0333); 2 - formation of the product amplification primer annealing at 50 ° C, 3 - formation of the product amplification primer annealing at 60 ° C; 4 - Formation of amplification product at the annealing temperature primers, 55 ° C.

The amplification product embedded into a plasmid PUC18 of restriction sites for *Nde* I and *Eco* RI, (neb.com/ restriction enzymes), which showed that the nucleotide sequence of the amplified fragment is equal to a full sequence of the gene bovine leukocyte α-interferon in the database.

Amplification product by restriction endonuclease sites *Nde* I and *Eco* RI embedded into plasmid pUC18. In lining prevents gene expression and protein formation [11]. Hybrid DNA transformed cells *E. coli* XL Blue. Selection of clones carrying the plasmid gene bovine interferon produced on the selective medium Eosin methylene blue(EMB). The presence of inserted bovine interferon gene in the plasmid pUC18 was tested. PCR analysis using primers F1 and R1, complementary to the 5'-and Z'-terminal sequences of bovine interferon gene (Figure 1), and restriction analysis figure-2.

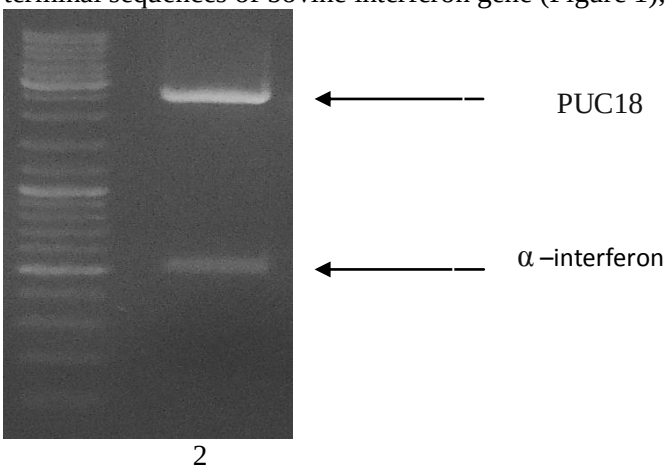

Figure 2- Electrophoresis results restriction Analysis Note: Track 1 - molecular weight marker Fermentas SM0333, lanes 2- plasmid pUC18, restriction enzymes *Nde* I and *EcoR* I.

The amplification product is embedded into a plasmid PUC18 the sites for the restriction *Nde*I and *EcoR* I. Then sequencing, which showed that the nucleotide sequence of the amplified fragment is equal to a full sequence of the gene bovine leukocyte α-interferon in the database.

In the next stage of the gene bovine leukocyte α-A-interferon transferred into vector pET24b$^{(+)}$ for restriction endonuclease sites *Nde*I-*Eco* RI.

The resulting recombinant plasmid, named respectively pM10952, transformed into *E. coli* XL-1 Blue. The choice of this system dictated by the plasmid-strain potential negative impact of the new animal protein in the bacterial cells, which could influence the results of, for example, the hypothetical case fatality rate of new protein in bacterial cells. The selected cells tested for transformation and inheritance of the plasmid characterized by restriction analysis of its properties. All plasmids contained an additional DNA fragment size corresponding to the gene of bovine α-A-interferon in figure-3.

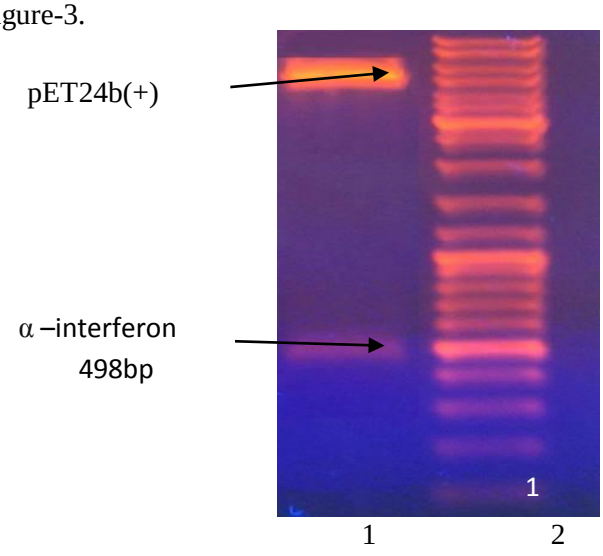

Figure 3- Electrophoresis results restriction Analysis. Line, lines 1- plasmid pET24b (+), processed restriction enzymes *Nde* I and *Eco*R I. 2 - Molecular weight marker Fermentas SM0333.

Conclusion:

Gene bovine leukocyte α-A-interferon amplified by means of PCR, using specific primers, on a matrix of a total DNA, which was isolated from cow blood. The gene was then cloned into the vector pUC18, pET24b (+) and sequenced. All plasmids contained an additional DNA fragment size corresponding to the gene of bovine α-A-interferon.

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