

Cloning and gene expression equine leukocyte α -interferon in cells of *Escherichia Coli*

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Abstract

Interferon's plays role in innate immune responses through upregulation of costimulatory molecules and induction of proinflammatory cytokines. Interferons including interferon alpha (IFNA). The present study characterized IFNA cDNA and predicted protein. The interferon's play a great role in protection from infections, which have been called by microorganisms, and also have powerful antiproliferation and immunomodulation activity. The purposes of study: cloning and expression of horse leukocyte interferon and purification of the product protein. The results and discussion: In the result we isolated (DNA) from equine leukocyte in blood, which was using in the quality of matrix for amplification of α -interferon gene with PCR HELP, and isolation gene α -interferon and transformation in vector puc18 and expression vector PET24b (+) and recombinant plasmid was transformed into *E. coli* strain BL21 (codon plus 440) induction with IPTG. The results showed the protein having the same molecular weight as horse interferon alpha about 18.5 kDa.

Introduction

In interferon (IFNs) were discovered in 1957 as a biological agent intervention in viral replication. They are mixtures of the secrets of proteins occurring in vertebrates and can be classified as cytokines. Interferon's are multifunctional components of defense against viral and parasitic infections and certain tumors. They affect the immune system function in various forms, interferon's perform various activities, primarily synthesize many proteins. 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. Interferon's are a class of glycoproteins that have antiviral,

antiproliferative and immunoregulatory activity (Wang H, Zhou M). There is three major types of interferon's: I and II and III interferon's. Type I form a large group of proteins that includes IFN alpha, delta, beta, kappa, omega, etc. Type II has IFN δ immune interferon, which has the interferon's and most mammals, is encoded by a single gene. Type II has IFN lambda (Kotenko, S. V.). The horse (*Equus Caballus*) found at least 6 genes encoding interferon alpha that is collected on chromosome 23. The aim of this study: cloning and subsequent gene expression equine α -interferon in the bacteria *E. coli* to obtain purified equine α -interferon may become the basis for the establishment of appropriate therapeutic and preventive veterinary drugs.

The Material and Methods

A. Bacterial strains and plasmids:
The bacteria strain *E. coli* XL-1 Blue (F'proAB lacIqlacZ Δ M15 Tn10 (Tcr) / recA1 endA1 gyrA96 (Nalr) thi-1 hsdR17supE44 relA1 lac) from the collection of the Department of Molecular Biology, Faculty

of Biology, Belarusian State University was used for cloning recombination plasmids. (Studier F. W., Rosenberg) The cells of *E. coli* BL21 (λ DE3) (hsd, gal, λ cIts857, ind1, Sam7, nin5, lacUV5-T7gen1), lysogenic for bacteriophage λ DE3,

containing the gene of bacteriophage T7 RNA polymerase under the control of PlacUV5-promoter in the cells of strain *E. coli* BL21-CodonPlus (DE3)-RIPL, containing amplified copies of the gene-Data is rarely encountered in prokaryotic m-RNA was performed inducible expression of target gene transcription in the bacteriophage T7.Plasmid pUC18 was used as a vector for the cloning of the gene sequence of horse α -interferon.Plasmid pET24b (+) was used as an expression vector.(Studier F., Moffatt B. A.)

B. Genetic-engineering techniques and enzymes:

Total DNA was isolated according to Construction(Mathew), isolation, restriction analysis of recombinant plasmid carrying ca +2 dependent transformation and

Results and discussion

Geneamplification equine leukocyte α -interferon with helps PCR used total DNA isolated from horse blood. At database GeneBank nucleotide sequences (codes M14540, M14541, M14542, M14543) primers were designed:

F1 (5-GGC CATATG TGT GAC CTG CCT CAC ACC CAT AGC CTG GGC-3 Select a

DNA electrophoresis was performed in under with generally accepted experimentprotocol. We used enzymes and buffer system firm MBI fermentas (Lithuania).polymerasechain reaction (PCR) (Frank C.)was performed in a mixture of standard composition with used programmable thermostat: Veriti (96well thermal cycle).Primers for PCR were designed on the basis of information from a database of nucleotide sequences of GeneBank. Sequencing was performed by the method of Sanger on the sequencer ALFExpress.Electrophoresis analysis of bacterial proteins was performed in 12% polyacrylamide gel under denaturing conditions with 0,1% SDS by the method. The gel was stained in a solution of Coomassie blue R-250.

site for restriction endonuclease *Nde*1) and R1 (5-gcg GAATTC GAT AAGCTT ACT CTG CTG CAA GTT TGT GGA TGA AGA GAA -3 allocated sites for restriction enzymes *Eco* R1 and *Hind*III) product size amplification equine interferon gene is (483) bp that is fully consistent with the expected (figure 1).

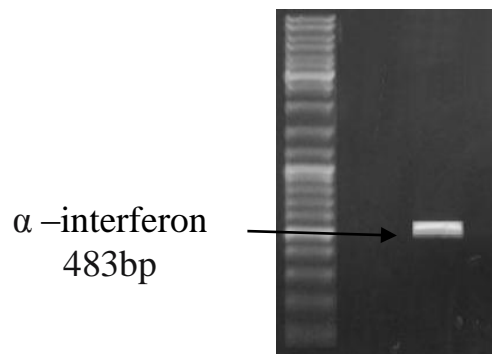


Figure 1. Gene equine leukocyte α -interferon

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

restriction endonuclease sites *Nde* I and *Eco* RI embedded into plasmid pUC18.Takoe inlining prevents gene expression and protein formation, which according to the literature can (Sambrook) to degrade bacterial intracellular proteases *E. coli*, or have lethal effect on cells of the new owner. Hybrid DNA transformed cells *E. coli* XL Blue. Selection of clones carrying the

plasmid gene equine interferon produced on the selective medium EMB. The presence of inserts equine interferon gene in the plasmid pUC18 tested PCR analysis using primers

F1 and R1, complementary to the 5'-and 3'-terminal sequences of equine interferon gene (Fig. 1), and restriction analysis (Fig. 3).

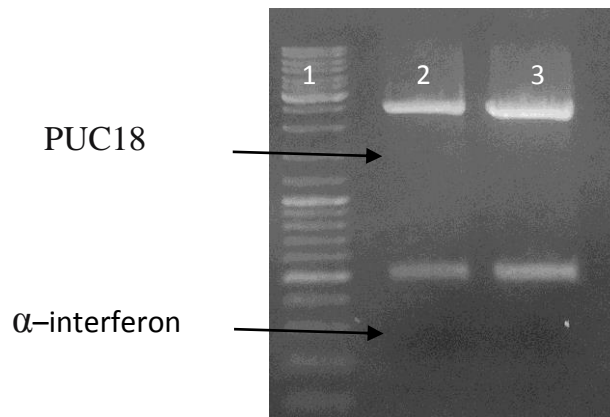


Figure 3. Electrophoresis results restriction Analysis Note: Track 1 - molecular weight marker Fermentas SM0333, lanes 2,3- plasmid pUC18, processed restriction enzymes *Nde* I and *Eco*R I.

The amplification product is embedded into a plasmid pUC18 the sites for the restriction *Nde* I and *Eco*R I. then spent his sequencing, which showed that the nucleotide sequence of the amplified fragment is equal to a full sequence of the gene equine leukocyte α -interferon in the

database. In the next stage of the gene equine leukocyte α -interferon transferred into an expression vector pET24b (+) for restriction endonuclease sites *Nde* I-*Eco* RI. The resulting recombinant plasmids, named respectively M14541, (Figure 4)

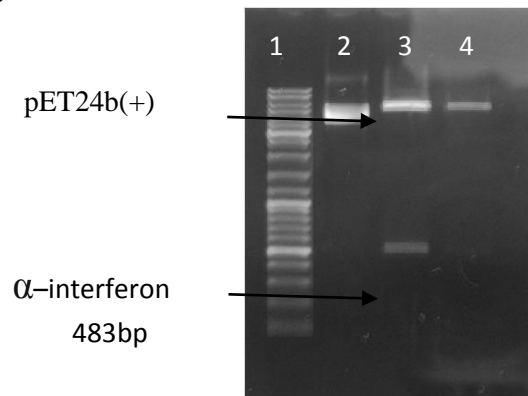


Figure 4. Electrophoresis results restriction Analysis Note: Track 1 - molecular weight marker Fermentas SM0333, lanes 2, plasmid pET24b (+), 3 - plasmid pET24b (+), processed restriction enzymes *Nde* I and *Eco*R I, 4-carved pET24b (+).

Transformed strain of *E. coli* BL21 codon plus 440 (Jerpseth), which is lysogenic for bacteriophage λ DE3, containing the gene of the RNA polymerase of bacteriophage T7 under the control of PlacUV5-promoter. *E. coli* cells VL21

codon plus 440, inherit these plasmids were grown in the presence of IPTG (isopropyl- β -D-thiogalactopyranosid) for induction of gene expression in equine leukocyte α -interferon (Fig. 5).

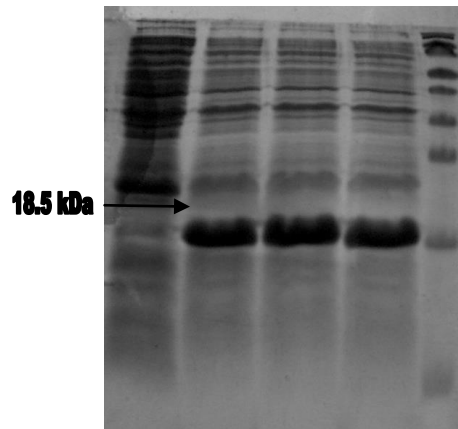


Fig.5.SDS-electrophoregram proteins of bacterial- cell proteins the bacteria *E. coli* BL21 condon plus 440 + pet24b + without IPTG.2,3,4 different proteins the bacteria *E. coli* BL21 condon plus 440 + pet24b + 4 hours after induction of IPTG at a co-ncentration of 1.0 mg / dL.5 - proteins of molecular weight standards (from top to bottom - 170 kDa, 130kDa, 95 kDa, 72 kDa, 55 kDa, 43 kDa, 26 kDa, 10 kDa) (Fermentas SM0671).

These results indicate that after the induction of IPTG in bacterial cells (Frank C.), containing the recombinant plasmid with the gene of equine leukocyte α -interferon, there is accumulation of the protein corresponding to the molecular weight of horse α -interferon (about 18.5 kDa).

On these results we can conclude:

- Using the designed specific primers amplification gene and the equine leukocyte α -interferon.
- Amplification product of the sequenced and cloned into the expression vector.
- Protein produced by size-fits to equine leukocyte α -interferon(about 18.5 kDa).

References

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استنساخ تعبير جين الانتزفيرون الفا من كريات الدم البيضاء للحصان في بكتريا القولون *Escherichia Coli*

عرفات عبد الرزاق مطر ماكسيم بتانوفج فلاديمير بروكليفيج
جامعة بيلاروس الحكومية - الأحياء المجهرية

الخلاصة

الانتزفيرونات تلعب دورا في الاستجابة المناعية الفطرية من خلال اعادة التنظيم وتحفيز السايوتوكاينيز من خلال تكوينها مضادات للفايروسات ومضادات التكاثر ولها دورا كبيرا في الحماية من العدوى بواسطة الكائنات الحية الدقيقة، ولها نشاط مناعي الغرض من الدراسة: استنساخ والتعبير الجيني لجين الانتزفيرون الفا وتنقية البروتين المنتج. النتائج والمناقشة: تم عزل (DNA) من الكريات البيض في دم الخيول، وتضخيم جين الانتزفيرون المعزول من الدنا بمساعدة PCR، ومن ثم التحول في ناقل puc18 تم التحول البكتيري بواسطة ناقل PET24b(+). البلازميد الهجين ينقل إلى سلالة الاي كولاي BL21 (440) والتحفيز بواسطة اضافة IPTG. أظهرت النتائج ان البروتين المنتج بواسطة بكتريا الاي كولي له نفس الوزن الجزيئي للانتزفيرون الفا للخيول بما يقارب 18.5 كيلو دالتون.