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Molecular cloning and sequencing analysis of the beta interferon from coturnix

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ABSTRACT

One pair of primers were designed according to chicken and meleagris gallopavo IFN-beta sequences published in GenBank.And the primers were used to amplify coturnix IFN-beta cDNA by RT-PCR from spleen of coturnix. The product was cloned into pEasy-T1 vector. Sequence was comparied with NCBI.We successfully got the coturnix INF-beta partial sequence. Sequence was subtyped and put up homologous analysis. The results suggested the homology of coturnix IFN-Beta gene and chicken and were 88.7%, 72.5% with platyrhynchos, 71.5% with meleagris gallopavo compared the nucleotide sequence in GenBank. The analysis of genetic tree showed that the relationship of coturnix and chicken IFN-Beta had high homology.

KEYWORDS

Coturnix;
IFN-B;
Gene cloning;
Gene sequence analysis.

INTRODUCTION

Interferon is a kind of highly active multifunctional glycoprotein, and it is an important cytokine with a broad range of biological activities.Interferon is secretory glycoprotein produced by the biological cells when it is subjected to the influence of the virus or other induce agent.Interferon has many kinds of bioactivity,such as antiviral activity, immune regulation and so on. Interferon is an important part of the body's defense system.Interferon has broad-spectrum resistance.When interferon acts on the body's organic tissue cell, can make it obtain the ability to resist a variety of viruses and microbes.Interferon has strict selectivity for a somatic cell, and has a relative species specificity.But the specificity is relative, not absolute.Interferon can be divided into I and II, interferon type I is the product of many gene families, including 14 -20 interferon alpha genes, 1 kind of interferon beta gene; interferon type II contains only one family member, namely the interferon gamma.

The meat and eggs of coturnix was delicious in China known as "animals ginseng". Currently number of coturnix raised is about 200 million in China, and 1/5 of the world. Coturnix is an important part of economic animal production in China. But the occurrence of avian influenza and New castle diseases were the serious threat to the production of coturnix. Establishing coturnix immune mechanism, looking for a new and efficient security immune route makes it necessary to coturnix breeding. By contrast, the reserch of animal interferon lags behind many. There is still the main stay in basic research and clinical trials, and most concentrating in a few animals such as pigs, chicken, fish. But it also made big progress in recent years. There are commercial interferon of pig, dog, chicken and recombinant interferon product come to market. However there are very

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few research reports about the coturnix interferon.

EXPERIMENTAL MATERIALS AND METHODS

Primary reagent

E.coli DH5αpurchased from Beijing TransGen Biotech Company, pEasy-T carrier purchased from Beijing TransGen Biotech Company, Reverse transcription kits, Xho I and EcoR restriction enzymes purchased from Fermentas Company, Gel Extraction Kit, GenEluteTM High Performance Plasmid Kits purchased from Shanghai Sangon Biological Company, Easy Tap plus DNA polymerase and DNA Marker purchased form Beijing TransGen Biotech Company. PCR Amplifier purchased from America ABI Company, DYY III-31A/31B electrophoresis tank purchased form Beijing Liuyi instrument plant, Gdldoc EQ Gel Imaging Analysis System purchased from America BIO-RAD Company, SUB28 Thermostatic Water Bath purchased from Britain Grant Company, Bechtop purchased from Canada Canadian Cabinets Company, Centrifuge 5417R Universal Intelligent table top refrigerated centrifuge.

Experimental method

Design primers and the amplification of cDNA sequence

One pair of primers were designed according to chicken and meleagris gallopavo IFN-beta sequences published in GenBank. The forward primer is atgactgcaaaccatcag,the reverse primer is tcactgggtgttgagacg. Coturnix spleen total RNA was extracted by Trizol method and was reversed transcribed to cDNA. Reverse transcription is processed with reverse transcription kit, reaction system is 10 μL. Blent reagent, put in the PCR Amplifier. 37°c15min, 85°c 5s, 4°c 5min. The rverse transcription product for PCR processed in PCR Amplifier, 94°c 5min;94°c 30s, 55°c 30s, 72°c 30min, 30cycles; 72°c 10min°c The products was detected amplification by 0.8% agarose electrophoresis.

Construction and identification of recombinant plasmid with IFN-β

Using of Gel Extraction Kit to purificat and recycle the PCR products. With reference to the instruction of cloning vector pEasy - T1, in order to join the components in the 10 ul reaction system. After gently blending, put it in the PCR instrument to connect 25 °c 10 min for conversion. Getting 5ul connecting product into fresh preparative DH5a cells,coating converted products on the LB/Amp plate evenly under aseptic conditions. Puting the tablet at 37°c thermostat in 12 h and picking a single colony after the culturing, preparing and evaluating plasmid DNA. Picking a single colony from the above overnight culture tablet randomly, vaccinating in 5 ml LB liquid medium containing Amp. Shaking culture overnight in 37°c,preparating plasmid DNA using GenEluteTM High Performance Plasmid Kits. Evaluating recombinant plasmid by PCR and restriction enzyme digestion. In order to further determine the resulting clone, recombinant plasmid identified as positive clones after the above steps was sent to Shanghai Sangon Biological Company for DNA sequencing. Sequencing results and the nucleotide sequence registered in GenBank was subtyped and putup homologous analysis.

IFN - beta nucleotide sequence homology comparison and the analysis of the evolutionary tree

Sequencing results of coturnix IFN-β and the nucleotide sequence of gallus (NM_001024836), Meleagris gallopavo (U28140), A.platyrhynchos (X84764), Mus musculus (NM_010510), Callithrix jacchus (AB571244), Ovis aries (NM_001009392), Danio rerio (NM_001261449), Papio anubis (NM_001173536), Macaca mulatta (NM_001042733), Homo sapiens (NM_000600), Mus musculus (NM_010510), Canis lupus familiaris (NM_001135787), Sus scrofa (NM_001003923), Oryctolagus cuniculus (NM_001082064) registered in GenBank was subtyped and putup homologous analysis.

RESULTS

Interferon gene cloning results

Total RNA was extracted from coturnix spleen cells, then amplified to products by RT-PCR.The PCR products was conducted electrophoresis in 0.8% agarose gel.There was a specific band in 400 bp with expecta-

tions (Figure 1).

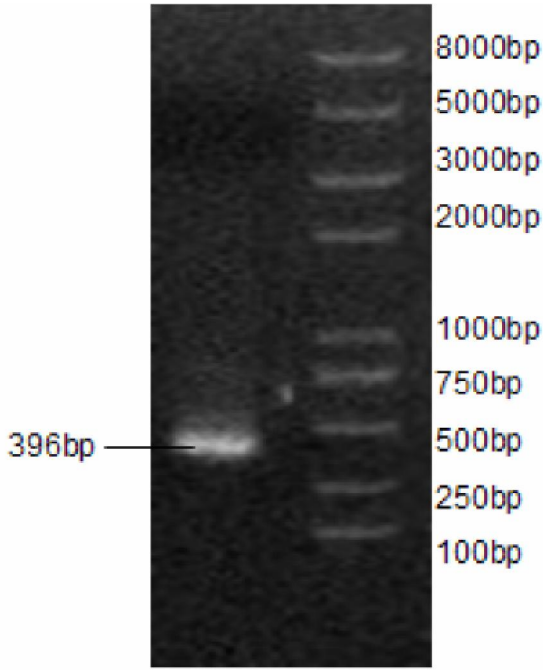

Figure 1: RT-PCR amplification of IFN-B

	1	2	3	4	5	6	7	8	9	10	11	12	13	
1		93.1	34.0	33.6	33.0	33.9	31.3	92.8	33.7	64.9	93.7	36.3	38.0	1
2	7.3		34.6	34.8	34.9	34.8	32.2	99.2	35.5	64.1	97.5	36.3	38.0	2
3	165.3	158.6		70.8	43.8	44.6	62.1	34.6	35.7	35.5	35.5	44.0	45.4	3
4	171.5	157.6	37.6		41.4	43.8	64.8	34.9	37.7	34.2	35.7	43.3	43.4	4
5	168.3	151.9	103.9	114.3		72.6	38.2	34.9	29.7	29.9	30.1	67.0	72.5	5
6	165.3	156.5	100.9	103.7	34.3		41.7	34.6	34.9	36.5	34.8	72.0	71.5	6
7	203.4	196.2	54.4	49.1	130.5	113.9		32.2	34.0	33.3	31.7	41.7	43.9	7
8	7.6	0.8	159.6	156.7	151.8	157.9	197.7		35.5	64.1	97.7	36.3	38.0	8
9	178.4	160.1	152.9	137.1	213.7	151.9	169.7	160.1		35.6	33.8	33.4	35.2	9
10	48.9	50.5	149.8	161.8	204.0	141.3	166.8	50.3	148.2		66.9	35.0	35.1	10
11	6.7	2.5	150.1	149.2	203.0	155.3	193.0	2.4	178.1	44.6		36.6	38.3	11
12	149.9	152.7	103.1	106.4	43.8	35.4	113.8	151.0	168.3	154.8	148.1		88.7	12
13	133.4	134.6	97.5	105.5	34.6	36.2	103.8	134.2	150.2	150.2	132.0	12.5		13
	1	2	3	4	5	6	7	8	9	10	11	12	13	

Callithrix jacchus.seq
Macaca mulatta.seq
Ovis aries.seq
Canis lupus familiaris seq
A.platyrhynchos IFN gene.seq
Meleagris gallopavo interferon, partial
Mus musculus.seq
Papio anubis.seq
Danio rerio.seq
Oryctolagus cuniculus seq
Homo sapiens.seq
gallus.seq
coturnix.seq

Figure 2 : Homology comparisons of nucleotide sequence among chickens, quails, ducks and other animals IFN-B from GenBank

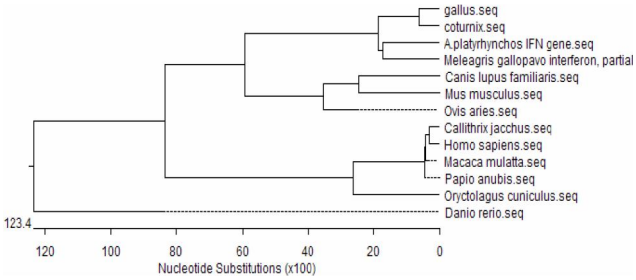

Figure 3 : The evolutionary tree of quail compared with other animals from Genbank

IFN-β sequence analysis

The homology of coturnix IFN-Beta gene and chicken and were 88.7% that shown in Figure 2,Figure 3, 72.5% with meleagris gallopavo and were 71.5% with platyrhynchos, 71.5% with meleagris gallopavo compared the nucleotide sequence in GenBankThe analysis of genetic tree showed that the relationship of coturnix and chicken IFN-Beta had high homology.

Figure 4 : Results of multiple alignments of amino acid sequences

	1	2	3	4	5	6	7	8	9	
1		10.7	13.9	11.2	15.0	98.6	10.2	12.2	9.8	1
2	476.0		20.7	62.6	9.8	10.7	49.7	19.6	19.1	2
3	357.0	238.0		19.0	10.0	13.9	21.7	53.4	78.8	3
4	447.0	51.5	258.0		10.4	11.2	62.0	17.4	19.8	4
5	328.0	524.0	517.0	493.0		15.5	8.2	7.4	10.2	5
6	1.4	476.0	357.0	447.0	317.0		10.2	12.2	9.8	6
7	504.0	80.6	226.0	52.5	688.0	504.0		20.1	22.1	7
8	407.0	252.0	71.1	284.0	819.0	407.0	244.0		56.8	8
9	524.0	258.0	25.0	248.0	504.0	524.0	221.0	63.3		9
	1	2	3	4	5	6	7	8	9	

Papio anubis.pro
Canis lupus familiaris.pro
gallus.pro
Sus scrofa.pro
Danio rerio.pro
Macaca mulatta.pro
Ovis aries.pro
Meleagris gallopavo.pro
coturnix.pro

Figure 5 : Homology comparisons of amino acid sequence among gallus, coturnix,and Meleagris gallopavo

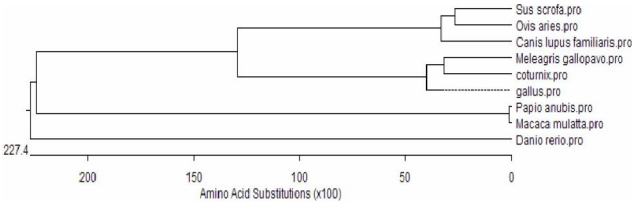

Figure 6 : The evolutionary tree of quail compared with other animals

IFN-βaminoacid sequence analysis

Can be seen from the above, the homology of coturnix IFN-Beta aminoacid and chicken and were 78.8%, the homology of coturnix IFN-Beta aminoacid and meleagris gallopavo were 56.8% by compared with the aminoacid sequence. The analysis of phylogenetic tree showed that the relationship of coturnix and chicken IFN-Beta had high homology.

CONCLUSION

We successfully got a coturnix INF-beta partial sequence. Sequence was subtyped and put up homologous analysis. The analysis of genetic tree showed that the relationship of coturnix and chicken IFN-Beta had high homology.

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REFERENCES

[1] A.Le Bon, G.D.Schiavoni, G.Agostino et al.; Type I interferons potenily enhanece humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo[J].Immunity, **14**, 461-70 **(2001)**.

[2] E.S.Clarles; Antiviral Actions of Interferons Clinical Microbiology Review[J]., **14(4)**, 778-809 **(2001)**.

[3] R.M.Roberts, L.Liu, Q.Guo et al.; The evolution of the type I interferons[J].J Interferon Cytokine Res, **18**, 805-816 **(1998)**.

[4] Ronit Shtrichman,E.S.Clarles; The role of gamma interferon in antimierobial immunity [J]. Current Opinion in Microbiology, **(4)**, 251-259 **(2001)**.

[5] K.Imada, J.W.Leonard; The Jak-STAT pathway[J]. Molecular Immunology, **37**, 1-11 **(2000)**.

[6] V.Ruvolo, L.Navarro, C.E.Sample et al.; The Epstein-Barrvirus SM Protein induces STATI and interferon stimulated gene expression[J].J Virol, **77(6)**, 3690-3701 **(2003)**.

[7] J.J.Rodriguez, L.F.Wang, C.M.Horvath; Hendra virus V protein inhibits interferon signaling by preventing STATI and STAT2 nuclear aceumulation[J].Virol, **77(21)**, 11842-11845 **(2003)**.

[8] Z.Wen, Z.Zhong, J.E.Damell; Maximal activation of transeription by Stat 1 and Stat 3 requires both tyrosine and serine phosphorylation [J].Cell, **82**, 241-250 **(1995)**.

[9] U.Ymkemeier, Moarefil, J.E.Damell et al; Structure of the amino-terminal protein interaction domain of STAT-4[J].Seienee, **279**, 1048-1052 **(1998)**.

[10] K.Takeda, T.Kaisho, N.Yoshida et al.; Stat 3 activation is responsible for IL-6-dependent T-cell prolifer-ation through preventing apoptosis:gene ration and characterization of T cell-specific Stat 3-deficient mice[J].J Immunol, **161**, 4652-4660 **(1998)**.