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Expression and purification of Murine IFN-γ protein from cloned *E. coli* strain containing pRSET A Vector with IFN gamma gene

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ABSTRACT

The cloned *E. coli* cell containing Murine IFN -γ inserted pRSET A vector system was effectively expressed in this study. The induction of the clones was done using IPTG in *E.coli* and induces mRNA generation and synthesis protein. It has shown an expression of protein with 18 kda in SDS PAGE and western blotting and their size was determined by GENE RUNNER software. This recombinant protein has a 6x His tag and it has been proved as it has shown a potent anti His property in western blotting. The purification of the protein was further done by Ni-NTA affinity chromatography. Nitrilo tri acetic acid (NTA) binds more stably with nickel (Ni) with 4 to 6 ligand binding sites in the coordination sphere of Nickel leaving two sites free to interact with the 6X His tag. The total results conclude that the targeted IFN gamma (408bp mouse gene) cloned in pRSET A was effectively expressed in *E. coli* BL21 strain cells and purified IFN gamma protein effectively as 1mg/ml. The purified IFN gamma protein may be used to diagnose the antiviral activity and antitumor activity.

Key words: IFN gamma, pRSET A, *E. coli,* SDS PAGE, Western Blotting

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1. INTRODUCTION

The interferon's (IFNs) are a cytokine and a large family of multifunctional secreted proteins involved in antiviral defense, cell growth regulation and immune activation [Lin and Young, 2012]. IFN-γ signaling is critical for the generation of effectors functions involved in the elimination of cancers and pathogenic microorganisms, the signalling must be carefully regulated to prevent excessive inflammation. The potential deleterious effects of IFN-γ signalling are also underscored by the fact that administration of IFN-γ to neonatal mice results in a lethal auto-inflammatory disease [Oberholzer, *et al.,* 2000]. These genes contain regulatory DNA sequences within their promoter regions Interferon-stimulated response element (ISRE), Interferon response element (IRS) that function as binding sites for a number of transcription factors and which are also expressed in response to other interferon [Lin and Young, 2012]. Gene cloning and vector construction are widely applied techniques in DNA and protein research and are the most frequently used technologies to study in vivo the role of the IFN system in the antiviral response [Nour Hammoudeh, *et al.,* 2015]. One of the most useful systems for expression of recombinant proteins in *Escherichia coli* is the pET vector series, which is based on the T7 phage RNA polymerase promoter and uses the pBR322 origin of DNA replication [Studier, *et al.,* 1990]. Improvement of vectors are being carried out so that they can be used for special purposes, such as for the synthesis of proteins that can be easily recovered and purified [Bhattacharaya, et al., 2005]. These studies have revealed, the possibilities of expression and purification of murine IFN gamma cloned *E. coli* containing pRSET A vector system.

2. MATERIALS AND METHODS

2.1. Confirmation of cloned *E. coli* strain BL21

The cloned *E. coli* was inoculated on LB media and incubated at 37^{0}C for two days. Afterward individual colonies were selected and streaked on agar plates containing 100µg/ml ampicillin and 35µg/µl chloramphenicol in a regular pattern and incubated at 37 ^{0}C for 16-18 hrs as per the methodology

of Kotenko, *et al*., [2008]. The expression of cloned pRSET A –IFN gamma were studied by colony PCR by following the methodology of Lim, *et al.,* [2003].

2.2. SDS-PAGE

SDS-PAGE was performed for analysis the cloned protein expression on *E. coli* cells by following the protocol of Krause, [2002]. The separated protein was visualised by staining the gel by 0.25% w/v coomassie Brilliant Blue (CBB –R 250, Himedia) stain in 45:10:45:methanol/acetic acid/ water on rocker for 2-3 hours. The gel was de-stained by placing the gel in water and changing the water several times until the background of the gel was clear. De-staining solution (Water with 20% methanol) was used when the background blue colour was not sufficiently removed with only water. Molecular weight of protein was determined as described by Larkin *et al*., [2000], by comparing to the standard molecular weight marker.

2.3. Dot Elisa

The standard Sambrook *et al.,* [1989] protocol was followed to study the expression of IFN gamma protein. The cell lysates were spotted as 4 circles on nitrocellulose paper. The protein-blotted membrane was washed with TBS solution and then blocked with 0.5% PBST for 1 hour along with primary antibody. The membrane was washed thoroughly with adequate volume of TBS and then incubated with anti-mouse antibody. Colour reaction was carried out with DAB and the result was documented.

2.4. Western blotting

The presence of IFN gamma transcribed protein expressed in the bacterial system was detected by western blot analysis as per standard procedure [Sambrook *et al*., 1989]. After SDS-PAGE electrophoresis the proteins were transferred on to nitrocellulose membrane using electrophoretic transfer cell of Biorad. The electrophoresed gel was washed twice in transfer buffer with 5 minute interval up to for 30 minutes. To transfer the protein separated by electrophoresis to NCM stack was prepared in the blotter unit consisting of Whatmann filter paper (3 layers), nitrocellulose membrane, electrophoresed gel, and three layers of Whatmann filter paper, arranged from cathode to anode and connected to power supply (60V) at 4 ^{0}C for 2 hours. After the transfer, the gel was removed and NCM was stained as mentioned earlier to check the transfer efficiency.

3. RESULTS

3.1. Confirmation of cloned *E. coli* with IFN-Gamma containing pRSET A Vector

The 408bp size amplified interferon gamma gene cloned with pRSET A vector in *E. coli* (competent) cells were obtained and confirmed by grown on ampicillin (100µg/ml) containing LB agar plates [Figure 1].

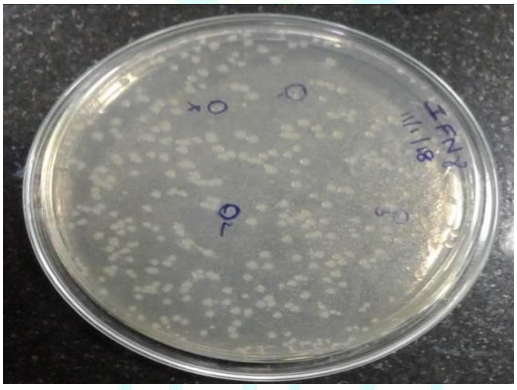


Figure 1 : Competent cells on LB agar plate

The induced IFN-gamma protein producing gene cloned vector was successfully expressed *E. coli* host (BL21pLysS). It was confirmed by using ampicillin (100µg/ml) containing LB agar plates and single pin point colonies were observed. The result has shows a LB agar plate in a single colonies.

3.2. Colony PCR of IFN-gamma in pRSETA vector

The amplified PCR product of IFN-gamma gene in pRSETA at *BamH*I and *Hind*III Sites was effectively confirmed on electrophoresis gel (Fig. 2). It confirms the amplified IFN-gamma was positively inserted with vector at exact restricted sites and ligated and transformed to get the cloned IFN-gamma genes in PRSET A vector. It shows an amplified IFN-gamma 408bp [Figure 2] on gel as Lane 1 to 4: Showing single band, amplicon of IFN-gamma 408bp from transformed colonies. L:1kb ladder

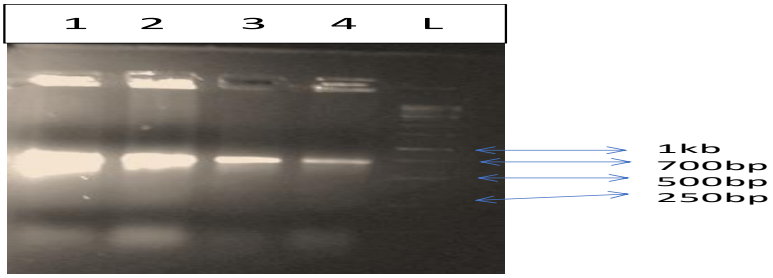


Figure 2: Agarose gel showing colony PCR for IFN-gamma [408bp] PCR fragment:

3.3. Expression of Fusion IFN-Gamma in *E. coli* Strains

The expression of cloned protein product from cloned *E. coli* was confirmed by SDS-PAGE and western blotting. In SDS-PAGE, uninduced sample and induced sample with IPTG have been loaded and both of these have shown an intense band in 18 kDa when compared with unstained protein ladder. 18 kDa is the actual size of the recombinant protein of IFN gamma. In western blotting it has shown an anti-His property and also has shown an intense band at 18 kDa [Figure 3] it confirmed the expression of IFN gamma protein from cloned *E. coli* cell.

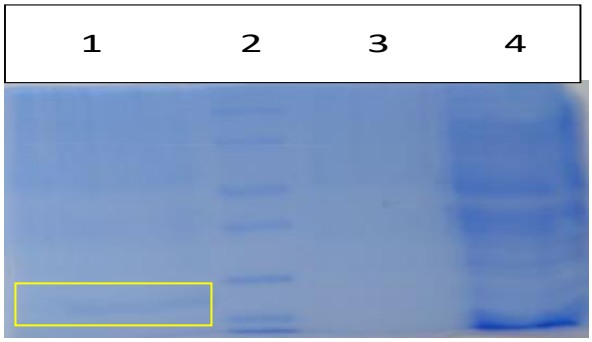


Figure 3: Purified IFN-gamma protein confirmed by SDS-PAGE:

1. IFN-gamma protein 18kDa 2. Unstained protein ladder 3. Uninduced IFN-gamma protein 4. Positive control

3.4. IFN-gamma expressed protein confirmed by Dot ELISA

IFN-gamma protein expression was confirmed by DOT ELISA (nitrocellulose membrane used) results [Figure 4]. Lane 1-IFN-gamma protein induced in pLysS, Lane 2-IFN-gamma protein induced inDE3, (lane 1 and 2 gives positive result) lane 3-positive control (His tag protein), Lane 4-uninduced IFN-gamma protein (no expression).

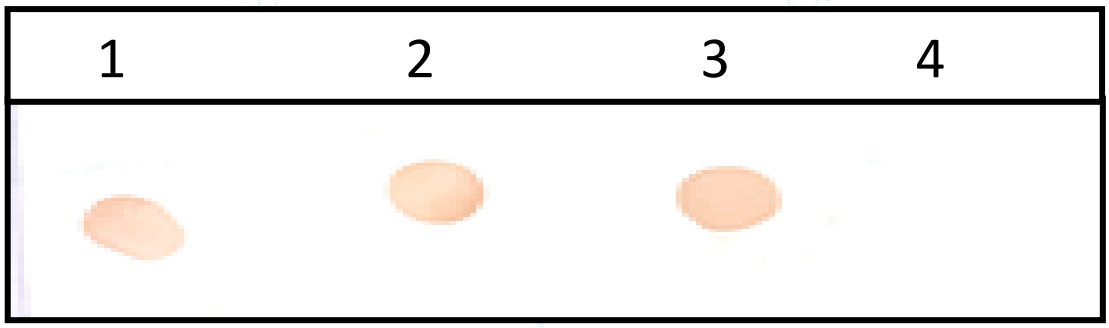
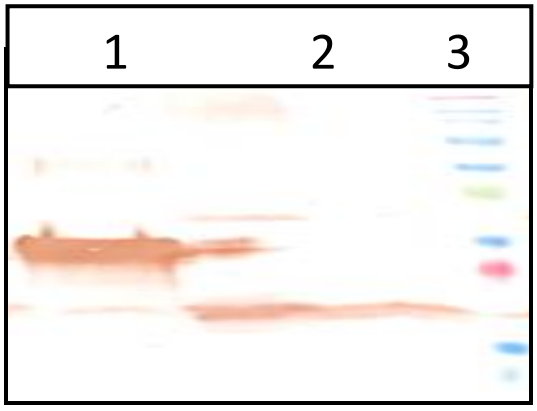


Figure 4:

1. Induced IFN-gamma protein in pLysS
2. Induced IFN-gamma protein in DE3
3. Positive control (His-tag protein)
4. Uninduced IFN-gamma protein

3.5. Western Blotting

The band and size of IFN-gamma protein on nitrocellulose membrane of western blotting was confirmed the expression and purified form of IFN-gamma protein expressed by IFN gamma gene inserted pRSETA vector on *E. coli* [Figure 5]. Lane 1-positive control protein, Lane 2-IFN-gamma protein induced in pLysS, Lane 3-IFN-gamma induced in pRSETA, Lane 4-prestained protein ladder.



1. Positive control
2. 2. IFN-gamma induced (pLysS)
3. 3. IFN-gamma protein (pRSET A)

Figure 5: IFN gamma on Western blotting

4. DISCUSSION

In the present study the IFN gamma gene of lymphocyte cells of female BALB/c mice cloned *E. coli* was utilized for finding the expression efficiency through pRSETA vectors and their purification possibilities. Similar kind of study was performed by various researchers. The expression of the recombinant protein using various plasmids are tightly regulated and transferred to effective host cells and obtained recombinant proteins (Oliveira, *et al.,* 2001). Several pET vectors have an affinity tag composed of six consecutive histidine residues (6XHis-tag) that permit the purification of the fusion protein on metal charged columns (Crowe, *et al.,* 1994). In contrast, the pRSETA expression system (Invitrogen, Carlsbad, CA, USA) is a high-copy number plasmid based on pUC19 origin of DNA replication that may confer a higher gene dose effect (Choi, *et al.,* 1999). In this, vector system, the proteins are produced with fusion of one leader peptide that encodes the first amino acids of the T7 gene 10 major capsid protein (T7-epitope tag), and the affinity 6XHistag. Lee *et al.,* [2000] utilized mouse interferon gene (MuIFN-γ), it was cloned and transformed to *Saccharomyces cerevisiae* and studied the expression in MuIFN-γ protein. They used two different promoters fused to MuIFN-γ were tested: glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter and a yeast hybrid ADH2-GPD (AG) promoter consisting of alcohol dehydrogenase II (ADH2) and GPD promoter. Using the hybrid promoter, the producer of recombinant MuIFN-γ and they got 0.2 mg/l. In the present study we confirmed the utilized cloned *E. coli* cell effectively expressed and it was confirmed by colony PCR results as 408bp sized IFN gamma gene from pRSET A vector (with hexa Histidine tag) of cloned *E. coli* cell. The BL21 *E. coli* strains of IFN gamma was purified in this study by using SDS PAGE and DOT ELISA and Western Blotting and obtained purified protein like 1mg/ml (18kda). Costa, *et al.,* [2016] has used human IFN gamma gene and expressed in *E. coli* and amplified by PCR and cloned in to recombinant vectors with hexa-histidine tag in Rosetta 2 (DE3) *E. coli* strain and obtained 0.8mg/ml of purified IFN gamma through anion and cation exchange chromatogaraphy. Pagliaccetti and Robek [2000], has used mice splenocytes cells for cDNA and used gene specific primers resulted in positive amplification of 468bp fragment. The presence of IFN-c fragment was confirmed by colony PCR, restriction endonuclease digestion and sequencing. Transformation and selection of IFN-c-pQE 30 in BL21 (DE3) pLysS strain was done and confirmed by SDS PAGE and western blotting and obtained 25kda size of protein. Marukian, *et al.,* [2006] have isolated mRNA from T lymphocytes cells and it was converted as cDNA using gene specific primers resulted 609bp. Further they used *E. coli* strain Rosetta (DE3) pLysS for the cloned cell preparation with hIFN-γ gene and proteins were confirmed by Western blotting and DOT ELISA.

5. CONCLUSION

The 408bp sized IFN gamma gene inserted pRSET A cloned *E. coli* BL21 (DE3) cells were effectively confirmed their expression. The expressed protein was initially confirmed by DOT ELISA and the size and purification was confirmed by western blot. The purified protein (18kda) was quantified by the nanodrop (1mg/ml). The quantified protein was confirmed by SDS PAGE. The purified IFN gamma protein may be used to diagnose the antiviral activity and antitumor activity.

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