RESEARCH ARTICLE

Production, Purification and Characterization of IFN-α2b in *Escherichia coli*

Ehab H. A. Mageed*, Falah H. Shary, Abdulelah A. Almayahi

College of Pharmacy, University of Basrah, Iraq

Received: 20th October, 2022; Revised: 27th October, 2022; Accepted: 20th November, 2022; Available Online: 25th December, 2022

ABSTRACT

IFNα-2b as a protein is a cytokine used to treat more than 14 diseases all around the world, the recombinant human IFN-2b was synthesized and a genetic engineering method or recombinant DNA technology. IFNα-2b as a protein with broad biological action, including antiviral and anticancer properties, might be a useful therapeutic protein for a variety of diseases, including hepatitis C and cancer. IFN-α2b sequence was taken from the National Center for Biotechnology Information (NCBI) gene database and optimized before being cloned and produced in the pET28a+ vector. *Escherichia coli* was considered as a prokaryotic expression system. IFN-α2b expression was carried out in *E. coli* strains BL21 (DE3) and BL21(DE3) pLysS and *E. coli* DH5α strain used for cloning. isopropyl-d-thiogalactoside (IPTG) induced protein production in bacteria cells bearing the pET28a+ IFN-α2b construct, followed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation of total proteins. Western blotting was used to confirm that IFN-α2b was expressed successfully in *E. coli* strains BL21 (DE3) and BL21(DE3) pLysS.

Keywords: IFNα-2b, Recombinant DNA, IPTG induction, SDS, Western blotting, *Escherichia coli*

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.4.27

How to cite this article: Mageed EHA, Shary FH, Almayahi AA. Production, Purification and Characterization of IFN-α2b in *Escherichia coli*. International Journal of Drug Delivery Technology. 2022;12(4):1640-1645.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Recombinant DNA technologies is helping to improve health outcome by creating new vaccinations and medications. Recombinant DNA technologies, genetics and gene treatment changes are frequently used in biotechnological and treatment diseases.[1] Many proteins that can be utilized for medical treatment or large-scale research are expressed using recombinant DNA technology, including antibiotics, hormones, cytokines, growth factors, vaccines, and many more proteins.[2] *Escherichia coli* the most widely used prokaryotic systems based on bacterial expression.[3] *E. coli* is the primary system used to manufacture a recombinant DNA product, allowing Eli Llly's DNA insulin to be approved since 1982.[4] Expressions protein in *E. coli* for various reasons: expressions the heterologous protein in *E. coli* host system is still the preferred method because it is lower coast that offers rapid growing cutler times and a high biomass and protein yields.[5]

Interferons (IFNs) are a type of cytokine that is induced when the host defense is challenged, and they are important for mobilizing the immune responses to infections.[6,7] IFNs is a 166-amino acid synthetic form of interferon.[8] IFNs are classified into alpha (leukocytes), beta (fibroblasts) and gamma (immune).[9] IFNs are classified into three categories based on their ability to: prevent viral replication in the host.[6]

IFNs are a type of cytokine that has antiviral, antiproliferative, immunomodulatory and antiproliferative immunomodulatory properties.[10] Interferons are classified into three classes based on their structure, functional properties and receptor specificity type I, II and III IFN.[8] Type-I interferon: includes IFN-α, IFN-β and IFN-ω. type I interferon binds to the same receptor known as the IFN-α receptor (IFNAR).[11] Type-II Interferon: has only IFN- γ that bind to IFN -γ receptors (IFN GR).[12] Type III Interferon: This type is represented by interferon lambda (IFN -λ).[12]

IFNs are signaling proteins that are capable to interfering with viral replications and play an important function in antiproliferative, antiangiogenic, immunomodulatory and anticancer activities.[13,14]

Interferons acts through (JAK-STAT) signal transduction pathway. The JAK-STAT pathway connected to MAPK founds in the eukaryotic cell, which controls a range of cell activitiy including, proliferation survival differentiations and apoptosis.[15] IFNα-2b is released by lymphocytes, macrophages and fibroblasts to stimulate the innate immune system's antiviral immunological response.[16] The molecular mechanism IFNα-2b as an anticancer effect, IFNα-2b has both direct and indirect antiproliferative activity.[15,14]

The IFNα- 2b a cytokine with a broad biological activity that

*Author for Correspondence: malugla@yahoo.com

is employed in the treatment of hepatitis and cancer.[15] The IFN-α2b also can be used to treat the human immunodeficiency virus (HIV) and various cancers, such as: malignant melanoma, chronic myeloid leukemia and renal cell carcinoma.[17]

Many *E. coli* strains are employed for recombinant DNA transformation protein productions. These strains include DH5α competent cells for cloning[18] and the BL21(DE3) competent cell for protein expression.[19] The *E. coli* strains BL21 (DE3) and BL21(DE3) pLysS inductions for producing recombinant proteins by using isopropyl-1-thio-β-D-galactopyranoside (IPTG) in order to induction T7 polymerase from the lacUV5 promoter are often used in inducer free expression vectors.[20,21]

This major purpose of the study to express human IFN-α2b gene into *E. coli* strains BL21 (DE3) and BL21(DE3) pLysS. The sequence was multi-optimized for *E. coli* expression.

MATERIALS AND METHODS

Bacteria Strains and Media

E. coli strain DH5α was used as the cloning host. In addition, the strains BL21 (DE3) and BL21 (DE3) pLysS, applied for the production and expression of recombinant proteins.

Luria broth (LB) media supplemented with appropriate antibiotics was used for culturing bacteria cells. In order to culture the bacterial strains. LB medium supplemented kanamycin with final concentration of 50 mg/L. For cloning procedures. *E. coli* strain DH5α was grown at 37°C with 180 rpm shaking.

The biological host *E. coli* BL21 (DE3) and BL21(DE3) pLysS was used to express IFNα-2b. The human gene was introduced into the pET28a+ commercial expression-inducible plasmid vector by using the *BamHI* and *XhoI* restriction enzymes.

TA-cloning

Amplification of IFNα-2b by the *Pfu* polymerase was carried out for cloning thymine and adenine end (TA) for the cloning purpose. This step was done to prepare the IFNα-2b fragment for insertion into pTG19-T vector by adding dATP to both sides of the fragment so that the terminal Ts sticky ends of the pTG19-T vector could be ligated.

Plasmid Constructions

Amplification of IFNα-2b by the polymerase was carried out for cloning thymine and adenine end (TA) for the cloning purpose. This step was done to prepare the IFNα-2b fragment for insertion into pTG19-T vector by adding dATP to both sides of the fragment so that the terminal Ts sticky ends of the pTG19-T vector could be ligated. Before cloning the gene into a pTG19-T, the optimum annealing temperature for amplifying the IFN-α2b sequence determined through gradient PCR. According to the result, a specific sharp band was obtained by using *Taq* DNA polymerase at 57°C during 30 seconds.

The commercially available vector pET28a+ (Novagen) was utilized in this study to express the target gene IFN-α2b.

The primary IFN α2b sequence was adopted from NCBI data bank according to the accession number NG-029154 and was codon optimized for optimum gene expression in *E. coli*. The designed fragment was subjected to be synthesized *via* General Biosystem Company (USA). For Polymerase chain reaction (PCR) amplification, a specific pairs of primers were designed as follow; IFN-For primer 5′ AACCATGGGCGGATCCTGTGATCTGCC and IFN-Rev primer 5′ AAGAGCTCGAGTGCGGCCGCGAATTCCTTAC. The TA-cloned target sequence was sub-cloned in the expression vector pET28a+ *via BamH*I-*Xho*I restriction sites.

All procedures of cloning, preparing competent cells and transformation methods were conducted according to the Sambrook and Russel protocol.[22] Total, purification of DNA (*e.g.* PCR products or DNA fragments containing overhang nucleotides) from agarose gel was carried out using the glass milk method.

IPTG Induction

Bacterial cells harboring the gene constructs in *E. coli* BL21 (DE3) and BL21 (DE3) plysS strains were culture into LB broth containing suitable antibiotics (100 mg/L kanamycin). The overnight cultures were diluted in LB broth at a ratio of 1:100 with half of the antibiotics used for overnight cultures and incubated at 37°C until the OD600 reached 0.5.

At this stage, 2 mL of each sample was taken as uninduced samples and IPTG culture was given a final concentration of 0.4 mM to activate the lac operon and induce protein synthesis.[23]

Protein Extraction

Bacterial protein extraction was carried out in accordance with Vaillancourt's methods, with a few modifications.[23] As a result, IPTG-induced recombinant proteins were centrifuged for 5 minutes at 4°C at 5000 rpm, and the supernatant was carefully decanted.

For soluble protein extraction, 0.5 mL native lysis buffer (containing 50 mM NaH_2PO_4, 300 mM NaCl, pH=8) was added to each sample, vortexed quickly, and kept on ice for 15 minutes. For 10 minutes, the samples were centrifuged at 8000 rpm at 4°C. The soluble fraction of the supernatant was transferred to a fresh tube.

The pellets from the previous stage were vortexed and centrifuged at 8000 rpm for 10 minutes with 0.4 mL (8M Urea, 100 mM NaH_2PO_4, 10 mM Tris-Cl, pH=8) of denaturing lysis buffer. The supernatant was transferred to a new tube as an insoluble fraction.

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis and Western Blotting

Bacterial total proteins were SDS-PAGE was used to separate the samples. After running gel, one SDS-PAGE was transferred in staining solution and another gel was subjected for western blotting. Therefore, protein samples transferred from SDS-PAGE to nitrocellulose membrane (GE Healthcare), at 50 V for 20 minutes and 250 mA for 30 minutes. This procedure was done using Mini-PROTEAN® Tetra cell apparatus (BioRad). Following the transfer step, it was incubated overnight in 5% skimmed milk. The next day the membrane was washed in appropriate amount of washing buffer (PBST) three times; each

time 5 minutes. In the next step, the membrane was incubated in PBST containing mouse anti-his monoclonal antibody in the ratio 1:6000 at RT for 2 hours. The washing step was carried out, and then the rabbit anti-mouse horseradish peroxidase secondary antibody was added (1:4000) to each well. After 2 hours of incubation, washing step carried out and DAB substrate added on the membrane.

RESULT AND DISCUSSION

The current work is an effort by a genetic engineer to manufacture that IFN-2b proteins using pET 28a+ and *E. coli* strains BL21 and BL21 (DE3) pLysS expression vectors as system to produce the recombinant protein. We focus on results and discussion of cloning, transformation, expression and the production of IFNα-2b. Codon optimization is a possibility that may result in a more compatible expression in the hosts' expression systems provided pET28+ gene constructs.

The amplified gene would be inserted into an expression vector, the sequence accuracy was important. We believe this step is critical and must be conducted dynamically since the type of reaction mixture, the efficiency of the thermal cycler and other reaction variables can all affect the annealing temperature.

Clones detected on selective agar plates (LB agar) were examined largely using colony analysis PCR as shown in Figure 1. Used templates to amplify human IFNα-2b by using forward and reverse specific primers. The was analysis on 1% agarose gel electrophoresis, initial confirmation of the T-vector harboring IFNα-2b (PTG19-T/IFNα-2b) by using templates to amplify human IFNα-2b by using forward and reverse specific primers. The was analysis on 1% agarose gel electrophoresis

Furthermore, first confirmation of the extracted plasmids from the positive clones produced by using of restriction endonucleases, as shown in Figure 2. The digestion of plasmid through *NcoI-EcoRI*, *NcoI-XhoI* and *BamHI-XhoI* restriction sites, resulted in the releasing fragments of 530 and 519 bp, respectively. This examination validated the cloning of IFNα-2b, and the gel-purified released fragments were employed in further cloning processes. PTG19-T is the designation given to the resulting T-vector containing an optimized IFNα-2b gene.

The purified IFNα-2b gene and the pET-28a+ plasmid was restricted by using two endonuclease restriction enzymes, each enzyme's recognition sites in the IFNα-2b gene separate

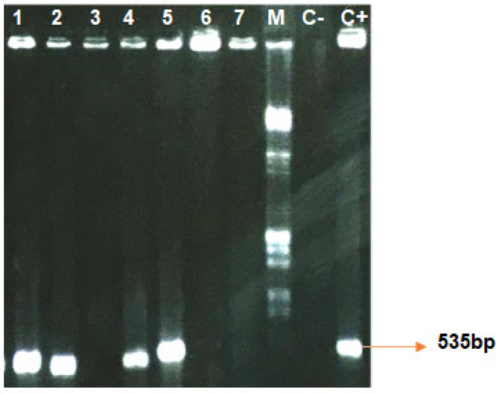

Figure 1: Using colony PCR, putative clones carrying PTG19-T/IFNα-2b were identified. The size bands (535 bp) of IFNα-2b gene on lane 1, 2, 3, 4, 5, 6 and 7 bands on 1% agarose gel of IFNα-2b gene. Negative and positive controls that indicate by letters C- and C+. Letter M represents a DNA ladder of 100 bp or more, visualized under UV light.

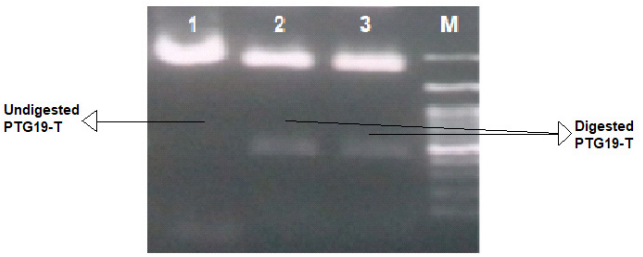

Figure 2: Confirmation PTG19-T vector containing the optimized IFNα-2b gene has been. The plasmid (PTG19-T) digested by *NcoI-EcoRI*, *NcoI-XhoI*, and *BamHI-XhoI* is represented by lane 1 and 2. The letter M indicates a DNA ladder of 100 bp or more, visualized under UV light.

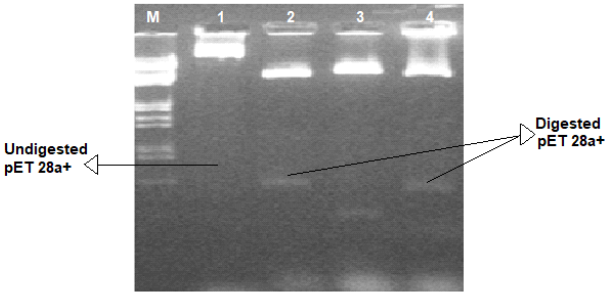

Figure 3: Confirmation of the gene construct IFNα-2b /pET28a+. Lane 1 indicates undigested pET28a+ plasmid as a control. Lane 2 and 4 (519 bp) indicate the plasmid isolated from a positive clone containing IFNα-2b/pET28a+ and digested with the restriction enzymes BamHI-XhoI (519 bp). The letter M represents a DNA ladder of 100 bp or more, visualized under UV light.

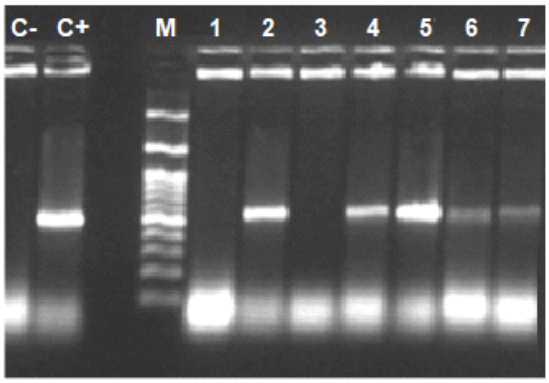

Figure 4: Colony PCR for screening clones grown on kanamycin agar plates. Lane 2, 4, 5, 6, and 7 Positive IFNα-2b fragments (549 b). Lane C+ positive control, Lane C- negatives control. The letter M represents a DNA ladder of 100 bp or more, visualized under UV light.

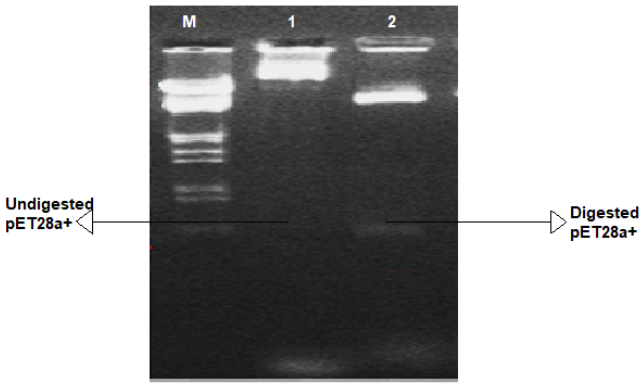

Figure 5: Confirming the validation of the IFNα-2b/pET28a+ constructed genes. The lane 1 indicates undigested pET28a+ plasmid as a control. Lane 2 indicate IFNα-2b /pET28a+ vector that is digested with BamHI-XhoI (the IFNα-2b; 567 bp). The letter M represents a DNA ladder of 100 bp or more, visualized under UV light.

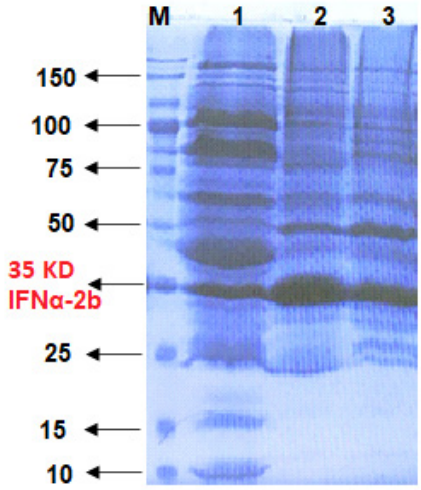

Figure 6: Bacterial expression of IFNα-2b by using *E. coli* BL21. Lane1 expressions IFNα-2b using *E. coli* BL21. Lan 2 and lan expression of IFNα-2b by BL21 (DE3). M represents the protein marker.

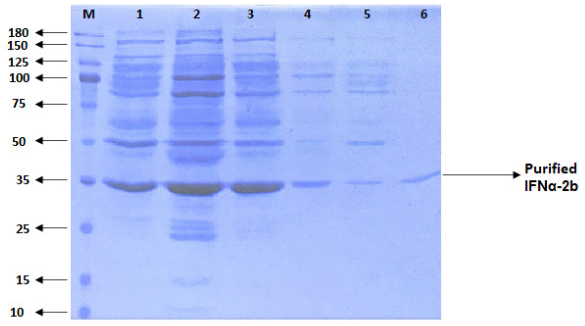

Figure 7: Purification of IFNα-2b based on His tags. Lane 1, 2 and 3 indicate soluble and insoluble fractions of recombinant protein IFNα-2b. Lane 4 and 5 showed early fractions came out from the column. Lane 6 indicates a purified IFNα-2b based on His tags. M represents the protein marker.

digestions of the IFNα-2b gene and the pET28a+ vector were performed by using *BamHI* and *XhoI* restriction enzymes.[24] Figure 3 shows the digestion result of IFNα-2b/pET28a+ using *BamH1* and *Xho1* restriction enzymes.

In this investigation, the restriction endonucleases *BamHI* and *XhoI* were chosen based on the map of the IFNα-2b sequence, so that these enzymes do not have a recognition site in the target gene. The primers were designed to include BamHI location in 5 end of a front primer and location *XhoI* in 5 end using reverse primer.

The transformation of recombinant IFNα-2b/pET28a+ plasmid, two *E. coli* strains were used. In biological science, the transformation of foreign DNA into host cells is a requires a specific procedure. Exogenous DNA molecules can enter a bacterial cell through natural or manufactured semi-permeability in to the cell wall.[25] The ability of a cell to absorb extracellular DNA from surroundings is referred to as cell competence. Cells with competence are naturally competent or unique ability of bacteria that are hypothesized in both laboratory condition[26] and the acquired expertise that increases when cells are cultured in a lab that treat make them DNA permeable. One among the most popular effective strategies for inducing competence in our isolates is to use $CaCl_2$ methods, which are more suitable for BL21 strains.[27] Mandel and Higa discovered this chemical method in 19/70 by introducing calcium chloride into a cells suspension which promotes the plasmid DNA that binds to the lipo polysaccharides. Positive charge Ca ions attach to the negative charge group between the inner cores of LPS and backbone of the negative charge of plasmid DNA, generating massive complexes that can't penetrate the membrane of a cell and go into the cytoplasm. The heat shocks step depolarizes cell membranes of $CaCl_2$-treated cells greatly due to the decrease in membrane potential, the cell's internal potential being negative decreases, resulting in migration DNA that is negative charge within system. The cold shocks restores the membrane of potential to its initial value.[28]

The effective ligation method that was confirmed by employing calcium chloride and heat shock for the transformation of *E. coli* DH5 α and of *E. coli* strains BL21 and BL21 (DE3) pLysS calcium is employed to generate competence in bacteria cell.[29]

The colony results for all transformed cells (*E. coli* DH5α) were shown of IFNα2b/pET28a+. Using a specific primer, a band of 549 bp was found on a % agarose as shown in Figure 4 and 5.

The expression of host *E. coli* strains BL21 and BL21 (DE3) pLysS are employed widely to express target protein in *E. coli* strains (pET system manual). Initially, both strains were employed in this study to see if there was any difference in their ability to produce the recombinant protein His -IFN-His.[20] Transformation of the IFNα-2b/pET28a+ constructs into *E. coli* strains BL21 (DE3) and BL21 (DE3) plysS showed no significant differences in protein expression between two strains, as shown in Figure 6.

The DH5α isolate genomic DNA is devoid of T7 RNA

polymerase that unable to make target gene active controlled by T7 promoters, the genotype of the DH5α strain has mutations that make it the optimum strain for laboratory cloning techniques.[30-32]

To obtain recombinant human IFNα-2b expression BL21 (DE3) isolates were transformed with a specially designed IFNα-2b/pET28a+ vectors were grown in a variety of growth media that were induced with IPTG as well as in auto-induction medium.

Several expression conditions were used in the current investigation to optimize the production of the IFNα-2b protein. The colonies containing the required gene fragment *E. coli* with IFNα-2b/pET28a+ in 5 mL medium containing 50 g/mL kanamycin as an indicator and 2% glucose as a support. The culture medium were then incubated for 8 to 48 hours at temperatures ranging from 18 to 37°C, with 0.4-1 mM IPTG employed to induce the production of the IFNα-2b protein.[33,34]

We used different concentrations of IPTG to reduce expression levels while increasing the solubility in *E. coli* BL21 cells which were recovered from inclusion bodies after mechanical lysis. Lowering the expression levels of target proteins may enhance their soluble yields.[35,36]

A recombinant protein's amino acid makeup or modification can affect its SDS-PAGE position.[37,38] The recombinant IFNα-2b generated as a fusion protein with an estimated molecular weight of (35 KD). Figure 6 n shows 35 KDa band on the SDS PAGE of human IFNα-2b protein.

After preparing a cell lysate from a cloning isolate that induces the production of recombinant fusion proteins, the latter was purified using affinity chromatography. The ability of the recombinant protein His-IFNα-2b-His to adhere to the His-trap HP columns allowed cell extracts from bacteria expressing IFNα-2b/pET28a+ to be purified successfully.

Figure 7 show lane 6 a purified IFNα-2b based on His tags, while lanes 4 and 5, There were non-specific attachments in early eluates, which is rare in this purification technique, but more pure proteins might be obtained with further optimization. The density of the isolated protein band on SDS-PAGE was estimated to be between 0.8 and 1-µg.

The recombinant protein (His-IFNα-2b -His) has a theoretical molecular weight about 24.4 kDa and a pI of 6.97. Furthermore, 10% SDS-PAGE separation of the total proteins, including IFNα-2b-His showed an expression band of approximately 26 kDa, theoretical M.W. 22.3 kD and a calculated p 8.21. Although the size of the observed expression bands on SDS-PAGE did not match the expected size of recombinant proteins, subsequent investigation, including western blotting

The recombinant protein (His-IFNα-2b -His) has a theoretical molecular weight about 24.4 kDa and a pI of 6.97. Furthermore, 10% SDS-PAGE separation of the total proteins, including IFNα-2b-His showed an expression band approximately 26 kDa, theoretical M.W. 22.3 kD and a calculated p 8.21. Although the size of the observed expression bands on SDS-PAGE did not match the expected size of recombinant proteins, the subsequent investigation, including western blotting, validated the expression see section (3-6). According to the studies, the difference between a protein's expected size and its position in an SDS-PAGE is unusual.[39]

The western blot is a popular method for detecting proteins and posttranslational modifications on proteins. Western blotting is a widely used technique for protein analysis that can provide semi-quantitative or quantitative data on the target protein.[40] Western blotting was used to identify the protein that was produced and secreted by bacterial recombinant IFNα-2b *E. coli* BL21(DE3) and *E. coli* BL21(DE3) pLysS strain, the primary confirmation of expressed IFNα-2b-His that was performed by using the anti-histidine monoclonal antibody.

As a result of the low amount of IFNα-2b-His expression in the host, a band of approximately 26 kDa has been identified. However, according to the western blotting results, the higher amount of protein induction achieved from other single colonies resulted in two bands, one for the monomer and the other for the dimer of target protein, as shown in Figure 8 and 9.

The use of antibody for the identification of recombinant IFNα-2b *E. coli* BL21. Lane 1: recombinant of IFNα-2b protein containing IFN-His., which represents the result of western blotting for bacterial total protein carrying IFN-His 26KDa. C-: negative control, C+: positive control of a known mixed sample of two purified recombinant proteins with 21 and 33 kDa, M: marker as a control.

CONCLUSION

The conclusions from the current study: Using cloning IFNα-2b to amplify gene on the plasmids isolated from transform bacteria cells, a gene-specific primer was used which is an excellent option to screen clone isolate. The heat shock method was used to successfully introduce the IFNα-2b/pET28a+ gene into *E. coli* BL21, resulting in genetically engineered strains carrying the IFNα-2b gene. IPTG can be used to induce the expression of the target protein in the induction medium. Western blot analysis showed IFNα-2b, which was produced into transforming bacteria successfully.

REFERENCES

1. Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, et al. Role of recombinant DNA technology to improve life. Int J Genomics. 2016.
2. Shanmugaraj B, Bulaon CJI, Phoolcharoen W. Plant molecular farming: A viable platform for recombinant biopharmaceutical production. Plants. 2020;9(7):1–19.
3. Rosano GL, Ceccarelli EA. Recombinant protein expression in Escherichia coli: Advances and challenges. Front Microbiol. 2014;5(APR):1–17.
4. Gopal GJ, Kumar A. Strategies for the production of recombinant protein in escherichia coli. Protein J. 2013;32(6):419–25.
5. Assenberg R, Wan PT, Geisse S, Mayr LM. Advances in recombinant protein expression for use in pharmaceutical research. Curr Opin Struct Biol [Internet]. 2013;23(3):393–402. Available from: http://dx.doi.org/10.1016/j.sbi.2013.03.008
6. Negishi H, Taniguchi T, Yanai H. The interferon (IFN) class of cytokines and the IFN regulatory factor (IRF) transcription factor family. Cold Spring Harb Perspect Biol. 2018;10(11):1–16.
7. Pestka S. The interferons: 50 years after their discovery, there is much more to learn. J Biol Chem. 2007;282(28):20047–51.
8. EL-Baky NA, Linjawi MH, Redwan EM. Auto-induction expres-

sion of human consensus interferon-alpha in Escherichia coli. BMC Biotechnol. 2015;15(1):1–10.
9. Motzer RJ, Bacik J, Murphy BA, Russo P, Mazumdar M. Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol. 2002;20(1):289–96.
10. de Padilla CML, Niewold TB. The type I interferons: Basic concepts and clinical relevance in immune-mediated inflammatory diseases. Gene. 2016;576(1):14–21.
11. De Weerd NA, Samarajiwa SA, Hertzog PJ. Type I interferon receptors: biochemistry and biological functions. J Biol Chem. 2007;282(28):20053–7.
12. Huang M, Jiang J-D, Peng Z. Recent advances in the anti-HCV mechanisms of interferon. Acta Pharm Sin B [Internet]. 2014;4(4):241–7. Available from: http://dx.doi.org/10.1016/j.apsb.2014.06.010
13. Sanlorenzo M, Vujic I, Carnevale-Schianca F, Quaglino P, Gammaitoni L, Fierro MT, et al. Role of interferon in melanoma: old hopes and new perspectives. Expert Opin Biol Ther [Internet]. 2017;17(4):475–83. Available from: http://dx.doi.org/10.1080/14712598.2017.1289169
14. Rafique I, Kirkwood JM, Tarhini AA. Immune checkpoint blockade and interferon-α in melanoma. In: Seminars in oncology. Elsevier; 2015. p. 436–47.
15. Asmana Ningrum R. Human Interferon Alpha-2b: A Therapeutic Protein for Cancer Treatment. Scientifica (Cairo). 2014:1–8.
16. Lewczuk N, Zdebik A, Bogusławska J. Interferon Alpha 2a and 2b in Ophthalmology: A Review. J Interf Cytokine Res. 2019;39(5):259–72.
17. Zhang Q, Wang C, Ma F, Yao L, Gao H, Zhu L, et al. Development and biological activity of long-acting recombinant human interferon- α 2b. 2020;1–9.
18. Taylor RG, Walker DC, McInnes RR. E. coli host strains significantly affect the quality of small scale plasmid DNA preparations used for sequencing. Nucleic Acids Res. 1993;21(7):1677.
19. Daegelen P, Studier FW, Lenski RE, Cure S, Kim JF. Tracing ancestors and relatives of Escherichia coli B, and the derivation of B strains REL606 and BL21 (DE3). J Mol Biol. 2009;394(4):634–43.
20. Joseph BC, Pichaimuthu S, Srimeenakshi S, Murthy M, Selvakumar K, Ganesan M, et al. An overview of the parameters for recombinant protein expression in Escherichia coli. J Cell Sci Ther. 2015;6(5):1.
21. Tran DTM, Phan TTP, Huynh TK, Dang NTK, Huynh PTK, Nguyen TM, et al. Development of inducer-free expression plasmids based on IPTG-inducible promoters for Bacillus subtilis. Microb Cell Fact. 2017;16(1):1–10.
22. Russell DW, Sambrook J. Molecular cloning: a laboratory manual. Vol. 1. Cold Spring Harbor Laboratory Cold Spring Harbor, NY; 2001.
23. Flowers JL, Vaillancourt LJ. Parameters affecting the efficiency of Agrobacterium tumefaciens-mediated transformation of Colletotrichum graminicola. Curr Genet. 2005;48(6):380–8.
24. Rohweder B, Semmelmann F, Endres C, Sterner R. Standardized cloning vectors for protein production and generation of large gene libraries in Escherichia Coli. Biotechniques. 2018;64(1):24–6.
25. Chang AY, Chau VW, Landas JA, Yvonne. Preparation of calcium competent Escherichia coli and heat-shock transformation. J Exp Microbiol Immunol. 2017;1(June):22–5.
26. Mell JC, Redfield RJ, Mell JC, Redfield RJ. COMPETENCE. 2014;196(January).
27. Chan W-T, Verma CS, Lane DP, Gan SK-E. A comparison and optimization of methods and factors affecting the transformation of Escherichia coli. Biosci Rep. 2013;33(6):e00086.
28. Das S, Dash HR. Microbial biotechnology-a laboratory manual for bacterial systems. Springer; 2014.
29. Inoue H, Nojima H, Okayama H. High efficiency transformation of Escherichia coli with plasmids. Gene. 1990;96(1):23–8.
30. Kostylev M, Otwell AE, Richardson RE, Suzuki Y. Cloning should be simple: Escherichia coli DH5α-mediated assembly of multiple DNA fragments with short end homologies. PLoS One. 2015;10(9):e0137466.
31. Al-Badran A, Abdul-Jabbar RA. Construction of Synthetic Intact Human Parathyroid Hormone Gene and Testing the Transformation Efficiency and Expression of it in E. coli strains. Am Sci Res J Eng Technol Sci. 2017;27(1):12–24.
32. Formulation and in vitro evaluation of valsartan flash tablet Sattar, M., Alshawi, M.A., Mosa, M.N. Systematic Reviews in Pharmacy, 2020, 11(10), pp. 213–219
33. Nikkhoi SK, Rahbarizadeh F, Ranjbar S, Khaleghi S, Farasat A. Liposomal nanoparticle armed with bivalent bispecific single-domain antibodies, novel weapon in HER2 positive cancerous cell lines targeting. Mol Immunol. 2018;96:98–109.
34. A comparative study of an in vitro release patterns of ceftaroline fosamil from chemically-prepared coated hydroxyapatite nanoparticles Alassadi, E.A.S., Jasim, E.Q., AL-Salman, H.N.K., Mosa, M.N.Systematic Reviews in Pharmacy, 2020, 11(3), pp. 797–805
35. Castro LS, Pereira P, Passarinha LA, Freire MG, Pedro AQ. Separation and Puri fi cation Technology Enhanced performance of polymer-polymer aqueous two-phase systems using ionic liquids as adjuvants towards the puri fi cation of recombinant proteins. Sep Purif Technol [Internet]. 2020;248(January):117051. Available from: https://doi.org/10.1016/j.seppur.2020.117051
36. Synthesis, characterization and antibacterial evaluation of oxoazetidin - Benzene sulfonamide derivatives as a hybrid antimicrobial agentsAli, A.T., Mosa, M.N., Alshaheen, Z.G., Muhammad Ali, M.A.Systematic Reviews in Pharmacy, 2020, 11(2), pp. 487–494
37. Shi Y, Mowery RA, Ashley J, Hentz M, Ramirez AJ, Bilgicer B, et al. Abnormal SDS-PAGE migration of cytosolic proteins can identify domains and mechanisms that control surfactant binding. Protein Sci. 2012;21(8):1197–209.
38. Synthesis, characterization and antibacterial evaluation of 1,3,4-oxadiazole derivatives Derawey, S.H., Mosa, M.N., Jasim, E.Q., Hraishawi, R.M.O.International Journal of Research in Pharmaceutical Sciences, 2019, 10(3), pp. 2342–2350
39. Guan Y, Zhu Q, Huang D, Zhao S, Jan Lo L, Peng J. An equation to estimate the difference between theoretically predicted and SDS PAGE-displayed molecular weights for an acidic peptide. Sci Rep [Internet]. 2015;5(April):1–11. Available from: http://dx.doi.org/10.1038/srep13370
40. Mishra M, Tiwari S, Gomes A V. Protein purification and analysis: next generation Western blotting techniques. Expert Rev Proteomics. 2017;14(11):1037–53.
41. Chemical modification and characterization of chitosan for pharmaceutical applications, Almualla, M.A., Mousa, M.N., Sattar, M. Egyptian Journal of Chemistrythis link is disabled, 2021, 64(7), pp. 3635–3649