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EXPRESSION AND PURIFICATION OF RECOMBINANT IFN-γ PROTEINS IN *ESCHERICHIA COLI* BL21

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ABSTRACT

Recombinant protein is a protein that it code was carried by recombinant DNA (r-DNA) i.e. two segments of DNA in plasmid. When r-DNA is inserted to bacteria, these bacteria will produce protein based on this recombinant DNA. This protein is called recombinant protein. The objective of present research study is to purify the recombinant protein from given unknown cellular culture. Molecular techniques like gene insertion, chemical transformation, Blue-white screening, Ultra-sonication, Electrophoresis purity test and Western blot identification test were followed. From the use of above molecular techniques, the recombinant IFN-γ was identified from *Escherichia coli* (strain BL-21). Present research paper also entails about the difficulties across during the production of recombinant IFN-γ proteins.

Keywords: Recombinant Proteins, r-DNA, Molecular techniques, IFN-γ, *Escherichia coli.*

INTRODUCTION

Recombinant protein is a protein that it code was carried by recombinant DNA (r-DNA). r-DNA means that two segments of DNA in plasmid. Plasmids are extra-chromosomal covalently closed circular (CCC) DNA molecules occurring in bacterial cells. When r-DNA is inserted to bacteria, these bacteria will produce protein based on this recombinant DNA.[14] This protein is called recombinant protein. Recombinant proteins from bacterial cell like *Escherichia coli* can be synthesized intracellularly by use of major five steps which are following:

- Isolation of the genes of interest.
- Incorporation of these genes into a transfer vector.
- Transfer of the vector to the organism to be modified.
- Transformation of the cells of the organism.
- Selection of the genetically modified organism (GMO) from those that have not been successfully modified.

The objective of present research is to identify the gene from given unknown cellular culture and express the desired isolated gene into the *Escherichia coli* for the synthesis of Recombinant proteins.[7]

MATERIALS AND METHOD

Following steps were used for the expression and identification of IFN-γ gene for the production of recombinant human IFN-γ proteins.

- **Chemical Transformation and Screening**

  Isolated IFN-γ gene was obtained from lymphocytes culture and inserted into *Escherichia coli* BL-21 as host cells. Chemical transformation method comprises of preparation of competent cells by Calcium chloride treatment. Calcium chloride transformation is a method of promoting competence. 50 µl glycerol stock, Luria Bertani (LB) broth, $CaCl_2$, 100 µl Ampicillin were used as

Reagents. Chilling cells in the presence of divalent cations such as Ca^{2+} (in $CaCl_2$) prepares the cell membrane to become permeable to plasmid DNA. Cells are incubated on ice with the DNA and then briefly heat shocked (e.g. 42 °C for 30-120 seconds), which causes the DNA to enter the cell. pET Expression System were used as Vector in host cells. Competent cells were transformed with IPTG (isopropyl-beta-D-thio-galacto-pyranoside) induction for screening of recombinant cells by Blue-white colony identification method. White bacterial colonies were recognized as Recombinant while the blue were non-recombinant colonies.[7]

- **Quantitative assessment**

  Acetone precipitation protocol was followed to precipitate out the components of unsuitable buffer in recombinant proteins samples. Acetone precipitation was done by addition of 1ml chilled acetone in samples and twice rounds of centrifugation at14000rpm for 4℃, 10 minutes. Bradford protein analytical assay was used to measure the concentration of proteins in a solution at absorbance 595nm.[7]

- **Identification and Purity Tests**

  Identification and purification of sample proteins was done by Western Blot technique and SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) method. Protein molecular weight marker is a mixture of purified proteins supplied pre-diluted with sample loading to an SDS-PAGE. The proteins resolve into sharp bands and visualized by commassie blue staining. After staining, place the gel in distaining solution and captured the better image of gel in gel-doc operator. Molecular weights of the loaded protein samples on gel doc and protein bands were read.[7] Detail information about the gene and its identifications were confirmed from molecular database available on PUBMED.

RESULTS AND DISCUSSION

Molecular weight 15,600 Dalton of recombinant human interferon-gamma (IFN-γ) proteins was found during this study. In human beings, the gene which codes for gamma-interferon (IFN-γ) is located in the 12th chromosome. Introns are non-coding region of gene, which are present on IFN-α and IFN-β gene but IFNG gene lacks introns. Structurally, interferons are part of the helical cytokine family and characterized by an amino acid chain that is 145-166 amino acids long. Recombinant proteins (r-IFN) can be separated by conventional column chromatography. To separate recombinant proteins from the cytoplasmic mixture is very difficult. Many challenges are here, like formation of inclusion bodies, secretion of bacterial endotoxins in proteinitious environment and mycoplasmic contaminations. These problems can be solved by Bacterial endotoxin assay and LAL- test (Limulus amebocyte lysate). Interferons are naturally occurring glycoprotein's secreted by the eukaryotic cells in response to viral infections, tumors, and other biological inducers. Interferons produce clinical benefits for disease states such as hepatitis, various cancers, Multiple sclerosis and many other diseases.

CONCLUSION

It is found in some earlier researches that r-IFN-γ proteins are useful in prevention of chronic granulomatous disease. Actimmune drug is suggested for the treatment of chronic granulomatous disease, Osteopetrosis. Binds directly to the type II interferon gamma receptor IFNGR1, leading to a complex of IFNGR1 and IFNGR2. This activates JAK1 and JAK2 kinases which form a STAT1 docking site. This leads to STAT1 phosphorylation, nuclear translocation and initiation of gene transcription of multiple immune-related genes. Actimmune® (IFN-γ1b) is a single-chain polypeptide containing 140 amino acids. Production of Actimmune® is achieved by fermentation of a genetically engineered *Escherichia coli* bacterium containing

DNA that encodes for the human interferon gamma protein. A bone marrow transplant is the only known cure for Osteopetrosis, although Actimmune® has been shown to slow progression of the disease. Slowing the disease can be particularly important in cases of malignant Osteopetrosis, because it can take time to find an appropriate donor for a bone marrow transplant. Other drugs also have shown a slowing effect on Osteopetrosis, but Actimmune® was the first drug with FDA approval for this treatment.

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