How to Cite:
Mutar, B. A. R., & Panchal, R. K. R. (2022). Cloning & expression and production SAK enzyme from staphylococcus aureus and transfer in bacteria E. coli. *International Journal of Health Sciences*, *6*(S4), 5970–5877. https://doi.org/10.53730/ijhs.v6nS4.10443

Cloning & expression and production SAK enzyme from staphylococcus aureus and transfer in bacteria E. coli

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***Abstract*---**Staphylococcus aureus is one of the most dangerous organisms that threatens the life of humans and animals alike. It has been found that people who suffer from chronic diseases such as diabetes, blood vessels, pulmonary and cancerous diseases are more susceptible to infection, in addition to ability resist antibiotics, which made fierce and dangerous. This staphylococcus aureus possessed the SAK gene, which acts as a bio stimulant that plays an important role in the process of analyzing blood coagulation, as it acts as an anti-plasmogen activator and is in the form of an equal compound of human plasmin. SAK consists of a chain of amino acids numbering 136 amino acids. The study aimed to isolate the SAK gene using amplification technique PCR and insert it into an ideal vectorPET21a. And he transferred it to one of the strains of the E.coli(BL21DE3) , stimulating the bacteria by adding IPTG, the purpose of obtaining the highest level of protein expression in an ideal time, then purifying the protein by affinity chromatography . The most important affinity mark is the polyhistidine residue. which depend on the imidazole histidine ring to easily form coordination bonds with a stable transition metal. found size SAK 15 kDa during the protein expression process.

***Keywords*---**cloning, expression, production SAK enzyme, staphylococcus aureus.

Introduction

The formative nature of Staphylococcus aureus and the opportunistic pathogens that it causes to livestock and humans have made it the focus of researchers' attention because of the threats it poses to life on Earth through the complex mechanism that it uses in the secretion of nanoparticles that make it resistant to all antibiotics and immunity (1,2). Staphylococcus aureus is a Gram-positive bacteria whose behavior differs from the rest of the bacteria in the mechanism of avoiding the host's defense systems by protecting the host's ketonemic antimicrobials, which helps them build and form biofilms on the vital materials of the infected organism to ensure its survival (3). SAK is one of the enzymes produced by Staphylococcus aureus bacteria, as this enzyme is produced by more than 100 strains of these bacteria. Moreover, the amount of production of this enzyme varies from one strain to another, where infections of the skin areas were found to be the most productive than others (4). Due to the important role that SAK enzyme plays in dissolving blood clots and its anticoagulant activity, it has become a subject of interest for clinical uses in elucidating the multiple roles of coagulases as well as through the fibrinolytic system (5) Staphylokinase which is also known as SAK, is produced by majority of Staphylococcus aureus strains. If such a protein is used as a fusion partner for the recombinant expression of a protein of interest, it can aid in purification process of recombinant protein. As staphylokinase enhances the solubility of are limited E.coli inherent proteins that are obtained in soluble fraction during expression, the yield of recombinant protein can be enhanced at reduced cost. Hence, Staphylokinase can be used as solubility tag for different non-glycosylated heterologous protein production which normally tends to get aggregated during expression. in addition to its ability to enhance its activity in tissue damage and improve invasion Bacterial (6) It acts as a bioactivator of plasminogen by facilitating the plasmin residues and precursors of the fibrin protease of the morphology, in which the binding of plasminogen is via special receptors present on the surface of the bacterial cell (7) BL21 (DE3) It is one of the strains of E, coli bacteria that is characterized by its cheap price and its ability to express high protein under in vitro conditions where it binds with the T7 promoter To provide the highest levels of protein expression of recombinant protein with inducer IPTG .(8-9).

pET Expression System and pET21a Vector

The plasmid (vector) pET21a was isolated from the culture of Escherichia coli DH5α carrying the plasmid by alkalolysis method. By using the protocol for the plasmid isolation kit in the methodology section. the cloning and expression of recombinant proteins in E.coli, pET system is most generally used. It is considered as one of the most important system. Under control of strong bacteriophage T7 transcription and translation signals, targeted genes are cloned in pET plasmids. T7 RNA polymerase gene is introduced in the modified E.coli host (BL21DE3) in front of LacUV5 promoter. When T7 RNA polymerase is fully induced by IPTG (11-12) . Recombinant production became one of the basics of the industrial revolution, which began in various fields in the pharmaceutical industry and knowledge of the behavior of living organisms. It was essential in most of the programs for discovering modern medicines that are small molecules over a period of thirty years (13).

Materials and Method

pET21a(+) Vector Plasmid Preparation

1. PET21a was a DNA vector from Novagen Sigma-Aldrich (India).
2. Plasmid Isolation using Qiagen kit which is designed based on Alkaline lysis method
3. Digestion Of pET21a(+) Vector with *Nde* I and *Hind III* Enzymes
4. Gel Extraction of Digested pET vector (Quigen Kit)
5. Polymerase Chain Reaction for Amplification of SAK gene Template DNA: Staphylokinase synthetic DNA synthesized by Integrated DNA Technologies (IDT), California, USA.
6. Purification of SAK PCR product (Agilent Kit)
7. Ligation of Digested SAK gene with digested pET21a(+) vector
8. Colony PCR for selection of recombinants carrying SAK gene
9. Recombinant pET21a(+) -SAK plasmid preparation
10. Digestion of pET21a(+) -SAK Vector with *Nde* I and *Hind III* enzymes
11. Transformation of competent DH5 alpha cells with pET21a(+) -SAK vector
12. Transformation of recombinant pET21a(+) -SAK plasmid in competent BL21(DE3)(*E.coli* expression host)
13. Fermentation Process (flask fermentation)
14. Protein profile by SDS-PAGE
15. Purification by chromatography infinity

Result

Amplification of Staphylokinase (SAK gene)

The amplification of the SAK gene was carried out using as a specific template by sequencing the primers within the PCR strategies through conditions suitable for the gene, where these procedures were carried out at 94 °C for 5 minutes at a time of 30 seconds and a duration of 72 °C and for another 30 seconds with another extension For 72 °C, the amplified gene was examined by electrophoresis (14-15).

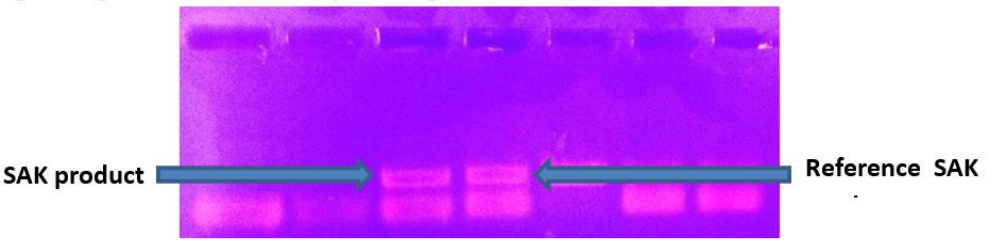

Fig.1:SAK PCR
Lane1: Negative Control. Lane2: SAK Product.Lane3: Reference SAK gene

Screen of staphlyokinase recombinant by colony PCR

We digested the SAK product by special enzymes are HindIII and Ndel with the vector digesting with the same digestion enzymes, ligating the pET21a transporter

and SAK amplicon from NdeI and HindIII using the ligase enzyme. The reaction was set up at room temperature as mentioned in the Materials section. Ligation was performed overnight at room temperature. DH5α-competent Escherichia coli cells were transduced with the bound product and the transformed cells were spread on LB + Amp agar plates. The plates were incubated at 37 °C overnight and the recombinants were obtained on The panels are shown in the figure above (16-17).

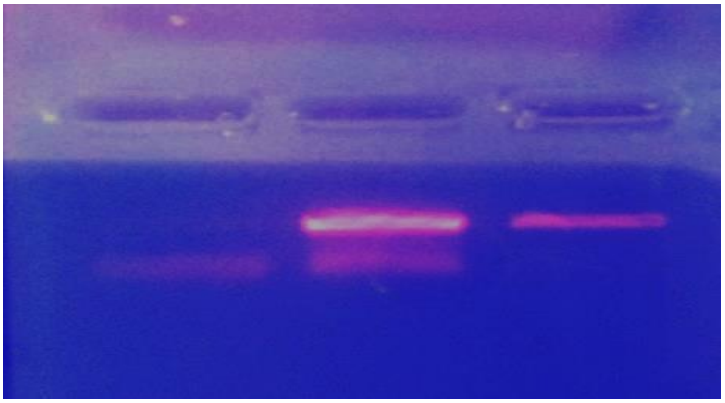

Fig.2 : Colony PCR Loading sequence:
1)Negative Control:10µL 2)Colony V-10µL 3) Colony No:1 -10µL 4) Colony No:2-10µL 5) Colony No:3-10µL 6) Colony No:4-10µL 7) Colony No.5:10µL.

Induction time optimization

(BL21DE3) cultures were used for three hours where we investigated by using IPTG, and again we used culture without the use of the inducer, post-induction cell Harvesting was done at different time intervals (half an hour and an hour to three hours) at 37°C, respectively to find the optimal induction time. At the end of the induction time, cells were detached by centrifugation. Cell pellets were suspended in 1.5 ml cold lysis buffer (10 mM Tris Cl pH 8.0), and disrupted by glass bead lysis in an ice bath. After lysis, the IB-dissociated fragments and the soluble fractions were loaded in 15% SDS-PAGE gels to visualize the touch protein profile(18).

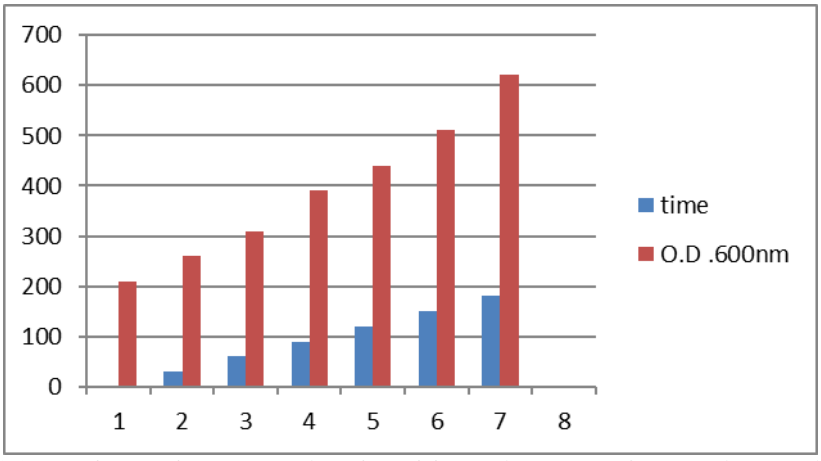

figure : (3) a culture that was stimulated by using IPTG for varying periods and measuring the density of the culture O.D 600nm

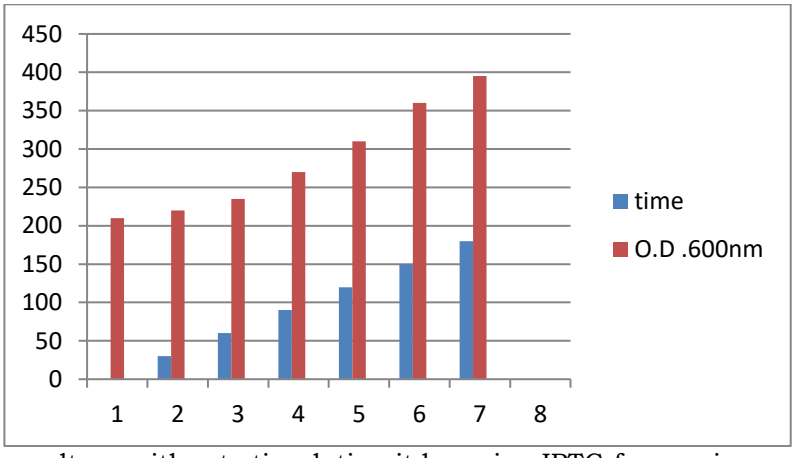

Fig: (4) the culture without stimulating it by using IPTG for varying periods and measuring the density of the culture

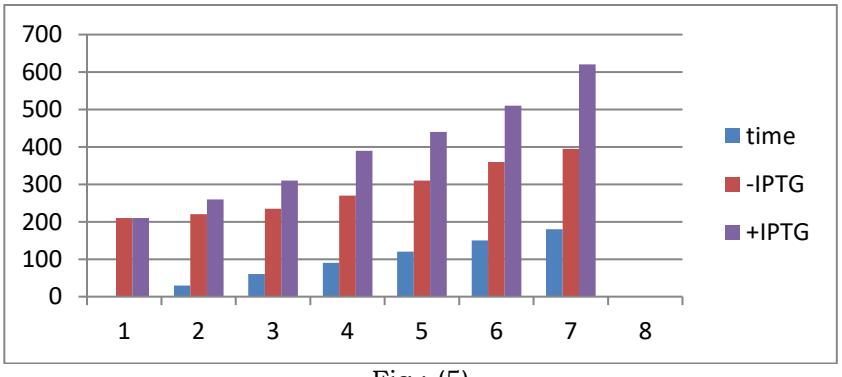

Fig : (5)
Comparing cultures when adding induction PTG and without adding PTG to culture BL21DE3

Expression of recombinant PET21a-SAK protein as analysed by SDS PAGE and stained with coomassie staining

We determined protein specifications using 15% SDS-PAGE. BL21DE3 expression culture host arrying recombinant pET-SAK-plasmid in LB + Amp medium for three h, induced with 1 mM IPTG for 3 h. At the end of induction, cells harvested and separated were soluble and insoluble fractions and loaded onto 15% lysis and 5% stacking. SDS-PAGE gel and stained with Coomassie stain. They were both granule fractions and supernatant Loaded with 20 µL and 40 µL, respectively. Together with a medium run marker 10µL (19).

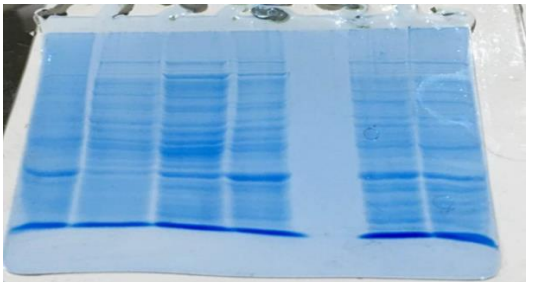

Fig:6 Expression protein inBL21(DE3) by induction IPTG

Affinity Purification Recombinant Protein in E.coli

Purification of the recombinant SAK protein was carried out using affinity chromatography using an imidazole ring with a histidine- tag and examined in 15% SDS PAGE Affinity Purification Recombinant Protein in BL21D(DE3)

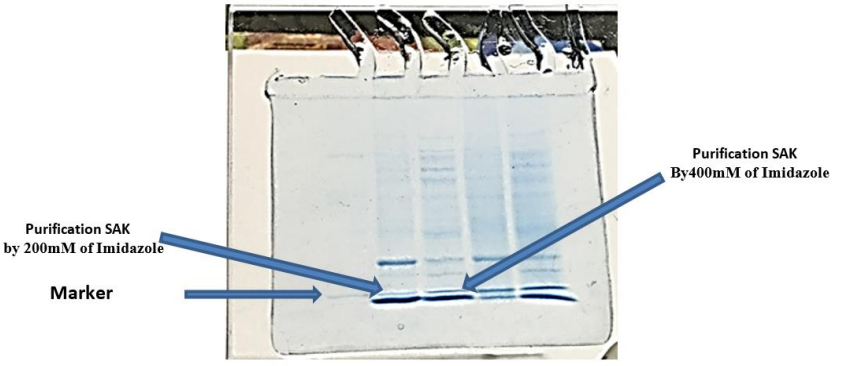

Fig 11: 15% SDS PAGE Affinity Purification Recombinant Protein in E.coli (Purification of Proteins Using Polyhistidine Affinity Tags) chromatography ; lane1 use marker ;lane2 original protein ; lane3 flow through ; lane4 wash purification protein ; lane5 elution by 200mM of Imidazole concentration ; lane6 elution by 400mM of Imidazole concentration ,

Dissection

The main objective of the research is the production of recombinant SAK protein, which is characterized by its plasmogen-degrading activity using the expression

system of Escherichia coli, being human friendly bacteria, which has made it play a major role in the biotechnology industry. With the PET21a carrier by HindIII and Ndel enzymes, after digestion is complete, we use the ligase enzyme to bind the gene to the carrier and incubate it for a while at room temperature, and then introduce it to BL21DI3, despite its presence(19). We encountered some problems in the expression process of coli genes, but the use of IPTG played a prominent role within the expression system, which helped to obtain the highest levels of protein expression in a timely manner, as we explained previously. For its stable metal affinity, it should be noted that this technique is widely used, especially with the host Escherichia coli, to produce soluble recombinant proteins within a host, which is characterized by cyclization to Ni by presence of high imidazole ring and ultimately we have obtained protein recombinant SAK(20) .

Conclusion

Despite the risk of gene expression which may pose a significant risk to the host as some of the recombinant proteins are highly toxic or may be insoluble and assemble into residues causing great damage, a recombinant sAC was produced whose size was 15 kDa which is a very small argument which It achieved a high melting rate of more than 70%.

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