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KEYWORDS:
Gene Cloning, Cloning vector, Competent cells.

Genetic Engineering of the Cloning Mutant Müller's Factor Gene Using Physical Mutagens of Alpha, Beta, Gamma Rays Emitted from CS137, CS138, Am241, Sr90, Sr91 and UV. Radiation Via Puc and M13 Cloning Vector Within Competent Bacterial Strain NM522 to Produce Recombinant Thrombolytic Enzyme Drug

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ABSTRACT:

Objective: The aim of the research is to cloning the Müller's factor gene after exposing it to various physical mutagenic agents from radioactive sources and ultraviolet radiation to increase production of thrombolytic enzyme with application in medical therapy such as thrombosis.

Backgrounds: S. aureus gram positive bacteria, produces many virulence factors such as hemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins, fibrinolytic enzyme and immune-modulatory factors. Thrombolytic enzyme is one of stronger factors released from S. aureus strains, expressed by lysogenic strains that degradation fibrin clot. It is known as thrombolytic enzyme, proteolytic enzyme, Staphylococcal fibrinolysin and Muller's factor.

Methodology: One hundred Staphylococcus aureus isolates were obtained from blood, tumors, cancer, pus, skin lesions and other sources. These isolates were exposed to various radioactive sources including Cs137, Cs138, Sr90, Sr91 and Am241 at different doses and for varying durations including alpha, beta and gamma radiation, also exposure to UV light at different time. The number of viable cells, the percentage of cell death, antibiotic activity and the production of thrombolytic enzymes were calculated before and after exposure. Polymerase chain reaction (PCR) was performed on the sak gene extracted from the mutant S. aureus. The PCR product was genetically engineered and the enzyme was extracted and purified from the bacteria in which the genetic engineering was successful. The lysis of blood clots was examined in vitro and also in mice.

Results and Discussions: The results of the antibiotic and fibrinolysis enzyme tests for the mutant S. aureus after exposure to alpha, beta, gamma and ultraviolet radiation showed that some of the bacteria that remained alive lost their resistance to antibiotics and became

very sensitive. The fibrinolysis enzyme production test showed that its production became many times greater than before exposure. The sak gene was extracted from all the mutant S. aureus and the product of the polymerase chain reaction was cloned into a genetically engineered bacterium NM522 using the M13 cloning vector and the pUC plasmid cloning vector. The results of the genetic engineering showed that 8 isolates of the genetically engineered NM522 bacteria had white colonies which is proof of the success of the cloning process. The enzyme was extracted and purified from genetically engineered bacteria. The clot formed in the laboratory was examined for human blood and the clot was examined inside mice. The results showed that the blood was restored to its liquid state and the clot was dissolved quickly within seconds. Likewise, inside mice, the blood was dissolved within a very short period after the process of inducing the formation of the clot was carried out inside the mice and examined after injecting the enzyme inside the mice.

Conclusions: The mutant bacteria transitioned from antibiotic-resistant to antibiotic-sensitive. The mutant bacteria transitioned from low or non-producing to producing a high level of the thrombolytic enzyme. The cloning process in genetic engineering successfully engineered the mutant gene from the mutant bacteria, resulting in the production of a highly effective thrombolytic enzyme for thrombosis and the treatment of blood clots and strokes.

INTRODUCTION

S. aureus opportunistic gram positive bacteria, released several virulence factors including toxins (superantigens, exfoliative toxins (ET), Toxic Shock Syndrome (TSST-1), Staphylococcalenterotoxin (SE) and cytolysins) and enzymes (coagulase and fibrinolytic enzyme, thermonuclese, leukocidins) and immune-modulatory factors [1]. It is a major pathogen in community-acquired and nosocomial infections [2].

Thrombolytic enzyme is one of stronger factors created from *S. aureus*, expressed by lysogenic strains that degradation fibrin clot [3]. It is termed thrombolytic enzyme, Muller's factor, proteolytic enzyme and fibrinolysin [4].

Protein engineering is the practicability for developing proteins or enzymes by manipulating for stability and specificity by utilize recombinant DNA technology in order to alteration in amino acid sequences [5]. Cloning vector is a DNA molecule ligated with foreign DNA, then transform into a host cell [6].

NM522 bacterial strain are cells qualified to receive foreign genes in genetic engineering. They are economical bacteria for cloning in genetic engineering. They are resistant to antibiotics, making them easy to select and they contain the F' plasmid required to produce ssDNA. Selection is based on the blue-white color of the strains [7].

The pUC plasmid clone vector comes in two types pUC18 and pUC19 consists of 2700 base pairs. It is widely used in genetic engineering due to its high yield production rate. Selection is based on its blue-white color with the successful clone in genetic engineering is clearly selected from the empty clone vector. It has three essential sites including origion of Replication (PMBi/ori), Ampicillin Resistance Gene (AmpR) and Multiple cloning site (MCS) Polylinker [8, 9].

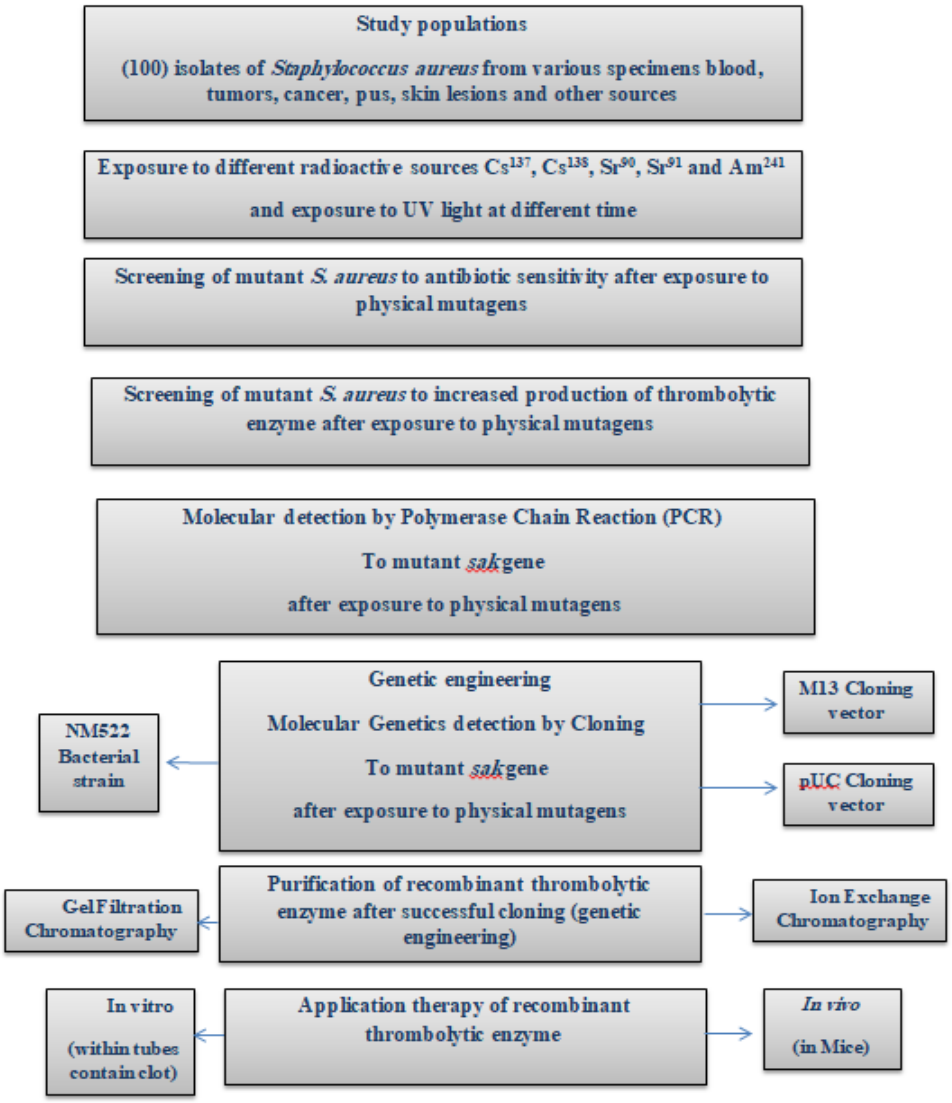
Application of thrombolytic Staphylokinase activity by lytic thrombi (Blood clot), thrombi concerning brain attack, peripheral artery disease, heart attack and venous thrombosis because its thrombolytic clot-dissolving trait (Plasminogen activator) [10, 11].

METHODOLOGY

One hundred *S. aureus* isolates were obtained from blood, tumors, cancer, pus, skin lesions and other sources. These isolates were exposed to various radioactive sources including Cs^{137}, Cs^{138}, Sr^{90}, Sr^{91} and Am^{241} at different doses and for varying durations including alpha, beta and gamma radiation, also exposure to UV light at different time. The number of viable cells, the percentage of cell death, antibiotic activity and the production of thrombolytic enzymes were calculated before and after exposure. Polymerase chain reaction (PCR) was performed on the *sak* gene extracted from the mutant *S. aureus*. The PCR

product was genetically engineered and the enzyme was extracted and purified from the bacteria in which the genetic engineering was successful. The lysis of blood clots was examined *in vitro* and also in mice.

Study design



Scheme (1): Methodology of research project

Study population

One hundred samples were collected and cultured on different media, initiall on Nutrient agar and broth at 37 ^{0}C for 24 hrs. and then on selective media like Mannitol Salt Agar. Identification by Vitek2-GP [12].

Screening for Thrombolytic enzyme

Quantitative Screening of Thrombolytic enzyme by Plasma agar plate assay

Plasma medium was prepared for the investigation and phenotypic detection of thrombolytic enzyme production in all *S. aureus* isolates by inoculating (5)µl of bacteria cultured in nutrient broth medium for 24 hr. at 37°C. Positive results were detected by the presence of white or cream-colored lysis zones around the pits on the solid agar medium after add plasma [13].

Semi-quantitative Screening of Thrombolytic enzyme by Skimmed Milk agar plate assay (Casein agar assay)

The Skimmed milk agar medium detect for thrombolytic enzyme production by taken 78.5 ml distilled water, subsequently added 12.5 ml of unsaturated skimmed milk, performed hole (5mm) by using Cork borer in the center of the agar. Added 0.1 ml of cultivated Nutrient broth, incubated for 18 hr. at 37°C. Positive results were detected by the presence of non-colored lysis zones around the pits on the solid agar medium after add skimmed milk [14].

Exposure to physical mutagens

1- Placed the bacteria in the middle of the liquid nutrient broth for a overnight until they reached the late logarithmic or stationary phase.

2-Prepare the bacterial suspension by comparing its turbidity with that of a MacCFrland suspension.

3-Exposing the bacteria inside these tubes to the sample chamber acertified radiation cell irradiator (containing radioactive sources) where alpha, and gamma rays are present.

4- Plating, The method of culture involves diluting the bacteria onto solid agar medium and then identifying the living cells by selecting the optimal mutation dose of bacteria after the production is determined morphologically on the culture medium plates, selecting high-yielding industrial or phenotypic mutants [15].

These isolates were exposed to various radioactive sources including Cs^{137}, Cs^{138}, Sr^{90}, Sr^{91} and Am^{241} at different doses and for varying durations including alpha, beta and gamma radiation, also exposure to UV light at different time. The number of viable cells, the percentage of cell death according to equation below:

Survival % = (CFU per mL of Irradiated Sample / CFU per mL of Non-Irradiated Control) × 100

Antibiotic sensitivity test

Kirby-Bauer Disk Diffusion method used to detect Antibiotic activity and the production of thrombolytic enzymes were calculated before and after exposure.

1- Pure bacterial inoculum Preparation 3-5 colonies (18-24 hr.) add to sterile saline or broth match the cloudiness of bacterial suspension against 0.5.

2- 0.5 McFarland turbidity Standard with bacterial Concentration to $1\text{-}2*10^8$ CFU/mL

3- Streak the swab to suspension on Muller Hinton Agar plate. Put the antibiotic Disks on the plate and incubation for 24 hr. at 37.

4-Read Results by ruler to measure diameter by inhibition zone around the antibiotic disks and compared to CLSI [16, 17].

Molecular detection

Genotypic detection of mutant *sak* by Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was performed on the *sak* gene extracted from the mutant *S. aureus* compared to before exposure to physical mutagens [18].

Table 1 : PCR Amplification Program of mutant *sak* gene .

Initial denaturation	Denaturation	Annealing	Extension	Final extension
95 °C for 5 min. 1 cycle	94 °C for 1min.or 30 sec	52 °C for 1min. 30 sec	72 °C for 1min. 30 sec	72°C for 10 min. 1 cycle
	35 cycle			

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Molecular genetics by genetic engineering

Gene Cloning and transformation for production recombinant drug therapy

Gene cloning achieved by using restriction enzyme BamH1 and EcoRI for digestion DNA of pUC and M13 Novel cloning vector with sticky ends and digestion mutant *sak* gene from mutant *S. aureus*, then ligated digested fragment mutant *sak* gene with pUC and M13 cloning vector have sticky ends, subsequently transformation for recombinant pUC and M13 vector within NM522 bacterial strain the results showed positive successfully for transformant clone of NM522 bacterial strain that selected on plasma agar medium contain 50 mg/ml ampicillin as a selectable marker for (8) isolates [19].

Purification of recombinant drug therapy

Eight isolates culture in 150 ml of brain heart infusion broth (BHI) and incubated at 37°C for 24 hr. for isolation of thrombolytic or fibrinolytic enzyme.

Determination of thrombolytic enzyme

1-Solutions for Concentration [20].

A. Coomassie Brilliant Blue G-250 Stain

It was prepared by dissolving 0.1g of coomassie brilliant blue-G-250 in 50ml of 95% ethanol, then 100ml of 85% phosphoric acid was added with agitation and the volume was completed to one liter with distilled water. The mixture was filtrated via Wattman filter paper (No.1) and kept in a dark bottle.

B. Tris-HCl Buffer

It was prepared by dissolving 0.3g of Tris-HCl in 100ml distilled water, pH was adjusted to 7.5.

Concentration was determined according to [20] **as follow:**

1. A standard curve of bovine serum albumin was carried out by using different concentrations from BSA stock solution.

2. Then 2.5 ml of Coomassie brilliant blue G-250 dye was added, mixed and left to stand for 2 min at room temperature.

3. The absorbance at 595 nm was measured; the blank was prepared from 0.1 ml of Tris-HCl buffer and 2.5 ml of the dye reagent.

4. A standard curve was plotted between the BSA concentrations against the corresponding absorbance of the bovine serum albumin.

5. The protein concentration of lipopolysaccharide sample was estimated by taking 0.1 ml of 1mg/ml lipopolysaccharide solution (dissolved in Tris-HCl buffer), subjected to the same previous addition and read the absorbance at 595 nm. The protein concentration was calculated from the standard curve.

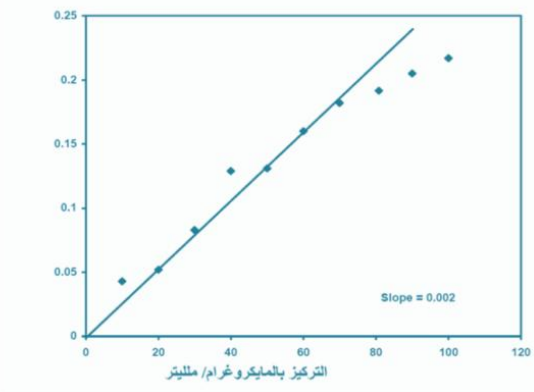

Figure (1): Standard curve for Bovine albumin protein.

Production and Purification recombinant thrombolytic enzyme

Production and Purification recombinant thrombolytic enzyme from recombinant NM522 bacterial strain clone via utilizing Ammonium sulfate, Diyalisis, Ion Exchange and Gel filtration chromatography for (8) transformant NM522 bacterial strain clone that express recombinant thrombolytic enzyme**.** The crude enzyme supernatant was fractionated by precipitation with ammonium sulfate between 50% and 70% of saturation. All subsequent steps were carried out at 4°C. The protein was resuspended in 20mM**.** Phosphate buffer, pH 7.0 and dialyzed against the same buffer, subsequently the partially purified enzyme was applied to anion exchange chromatography column packed with DEAE cellulose, bed volume of 25 mL**.**

Equilibrated with 20 mM phosphate buffer. PH 7.0. The bound proteins were eluted with a linear gradient of 0.05–0.5 M NaCl in 20 mM phosphate buffer, pH 7.0. The eluted fractions at the flow rate of 2 mL min-1were determined for SaK activity and protein concentration. Then the partially purified enzyme was then applied to Gel chromatography column packed with sephadex gel bed volume of 25 mL Equilibrated with 20 mM phosphate buffer. PH 7.0. The bound proteins were eluted with 20 mM phosphate buffer, pH 7.0. To determine for SaK activity and protein concentration.

Hydrolysis thrombi by recombinant thrombolytic enzyme

A- ***In vitro*** **recovery blood dissolve (within tubes)**

Determination of thrombolytic enzyme Activity was determined according to the modified Holmstrom method. In this method both purified SAK enzyme and ammonium sulphate precipitated samples were utilized. 1 ml of human blood were taken in eppendorf tubes and allowed to clot, subsequently recombinant thrombolytic enzyme was added at different concentrations [21, 22].

B- ***In vivo*** **(within Mice)**

Determination of thrombolytic enzyme Activity *in vivo*:

1- Prepare and anesthetize the mouse
2- Isolate the target vessel
3- Induce controlled thrombosis
4- Confirm vascular occlusion
5- Inject the thrombolytic enzyme
6- Measure recovery and recanalization

RESULTS AND DISCUSSIONS

Study population and exposure to radiation

One hundred *S. aureus* isolates from blood, tumors, cancer, pus, skin lesions and other sources. These isolates were exposed to various radioactive sources including Cs^{137}, Cs^{138}, Sr^{90}, Sr^{91} and Am^{241} at different doses ($1.776 * 10^{-4}$, 1.4157 and 63.100) and for varying durations (2, 3 and 4) hr. including alpha, beta and gamma radiation with viable cells from (10 to 15) cells and death percentage from (90 to 85) % to isolates number (5, 8, 14, 51, 52 and 62) as shown in table (2) and figure (2).

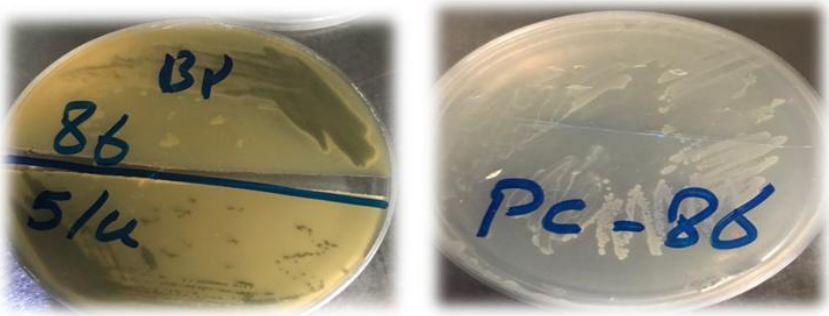

Figure (2): Mutant *S. aureus* exposed to physical mutagens.

Table (2): Exposing bacteria to different radioactive sources at different doses and times with calculating the percentage of bacteria killed.

Absorbance 490nm	Isotopes	Time exposure	Dose	Viable of bacteria	Killing of bacteria	No. of sample
Gamma 1	Cs137	4 hr.	1.776 *10-4	10	90%	5, 86
Gamma 2	Cs138	2 hr.	1.776*10-4	10	90%	86, 51
Alpha	Am241	3 hr.	1.4157	15	85%	8, 14, 52
Beta 1	Sr90	4 hr.	63.100	10	90%	5, 62, 51
Beta 2	Sr91	2 hr.	63.100	10	90%	5, 14, 62, 52, 51

Also exposure to UV light at different time (270, 180 and 150) minutes, also (5, 15 and 30) second, the number of viable cells (10, 15, 35,30 and 20) cells with percentage of cell death calculated from equation mentioned above (90, 85, 65, 7 and 80)% as shown in table (3).

Table (3): Exposing bacteria to UV. light at different times with calculating the percentage of bacteria killed and thrombolytic enzyme.

Absorbance 490nm	Time exposure	Viable of bacteria	Killing of bacteria	No. of sample	Diameter of thrombolytic enzyme on plasma agar before exposure to UV.	After exposure to UV.	Diameter of thrombolytic enzyme on plasma agar after exposure to UV.	After exposure to UV.
U 1	270 min.	10	90%	5, 8, 13, 17, 20, 52, 86	23	28	25	27
U 2	180 min.	10	90%	5, 8, 13, 17, 20, 52, 86	12	20	10	30
U 3	150 min.	15	85%	5, 8, 13, 17, 20, 52, 86	16	38	12	40
U 4	5 sec.	35	65%	5, 8, 13, 17, 20, 52, 86	16	24	22	25
U 5	15 sec.	30	70%	5, 8, 13, 17, 20, 52, 86	16	32	10	30
U 6	30 sec.	20	80%	5, 8, 13, 17, 20, 52, 86	0	29	0	24

Screening mutant bacteria and antibiotic sensitivity

All isolates of the mutant bacteria exposed to (CS^{137}, CS^{138}, Am^{241} ,sr^{90} , sr^{91} and UV. light became sensitive to all antibiotics which is proof of weakening the bacteria and reducing their resistance to antibiotics including Cox, Azm, CAZ, CC ,CN, CP,NOR, CRO, CX, E, FOX and IMP.

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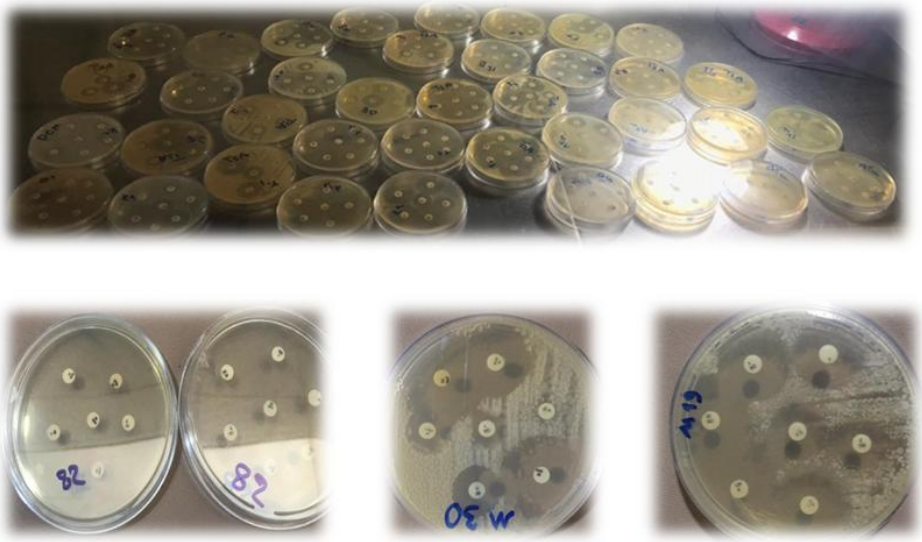

Figure (3): Antibiotic sensitivity test of mutant S. aureus exposed to physical mutagens.

The results of the antibiotic and fibrinolysis enzyme tests for the mutant *S. aureus* after exposure to alpha, beta, gamma and ultraviolet radiation showed that some of the bacteria that remained alive lost their resistance to antibiotics and became very sensitive as shown in figure (3).

Screening of mutant Thrombolytic enzyme

Screening for thrombolytic enzyme by quantitative assay on plasma agar plate and Semi-quantitative assay on achieved for mutant *S. aureus* in order to limit proteolysis on this medium.

The results give positive results by fibrinolysis zone around well on plasma agar and casein agar plate, this indicates for ability *S.aureus* for plasmolytic activity. The fibrinolysis enzyme production test showed that its production became many times greater than before exposure as shown in table (4), (3) and figure (4) .

Table(4): Number of fibrinolysis zone of thrombolytic enzyme around well

No. of Isolate	Fibrinolysis Zone(mm) before cloning	Recombinant Fibrinolysis Zone(mm) after cloning
1	15	20
2	17	23
3	0	4
4	18	22
5	14	19
6	0	2
7	0	3
8	18	20
9	15	20
10	14	20

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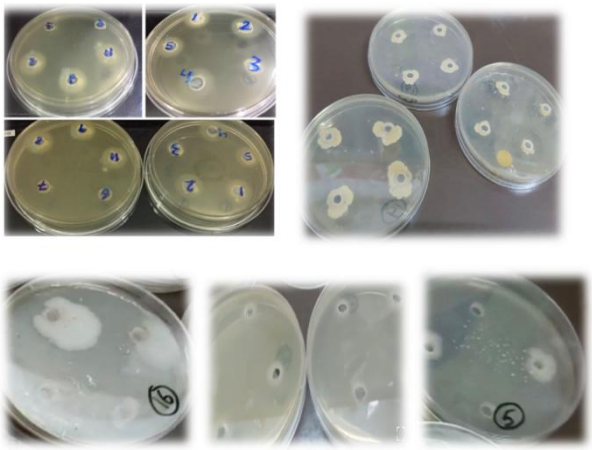

Figure (4): Number of fibrinolysis zone of thrombolytic enzyme around well

Molecular detection of PCR and genetic engineering
Gene cloning to produce recombinant drug therapy

The mutant *sak* gene was extracted from all the mutant *S. aureus* and the product of the polymerase chain reaction with size (900) bps after cloned into a genetically engineered bacterium NM522 using the M13 cloning vector and the pUC plasmid cloning vector. The results of the genetic engineering showed that (8) isolates of the genetically engineered NM522 bacteria had white colonies which is proof of the success of the cloning process. The enzyme was extracted and purified from genetically engineered bacteria.

Gene cloning achieved by utilizing restriction enzyme BamH1 and EcoRI for digestion DNA pUC and M13 Novel cloning vector with sticky ends and digestion *sak* gene *S.aureus*, then ligated digested fragment *sak* gene with pUC and M13 cloning vector have sticky ends, subsequently transformation for recombinant pUC and M13 vector within NM522 bacterial strain, the results showed positive successfully for transformants clone NM522 of that selected on plasma agar medium contain 50 mg/ml ampicillin as a selectable marker for, the result exhibit (8) isolates capable to growth in presence of ampicillin and producing recombinant thrombolytic enzyme serve as recombinant drug therapy for hydrolyze thrombosis as shown in figure (5).

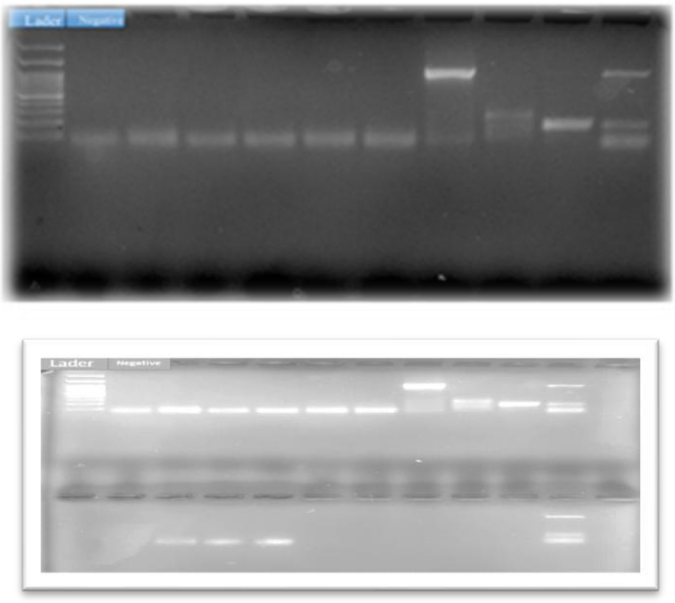

Figure (5) : Gel electrophoresis for detection amplified recombinant pUC and M13 Cloning vector (900bp) on agarose gel (1%), 50V for 1 hr., (M) DNA Ladder (1500 bp).

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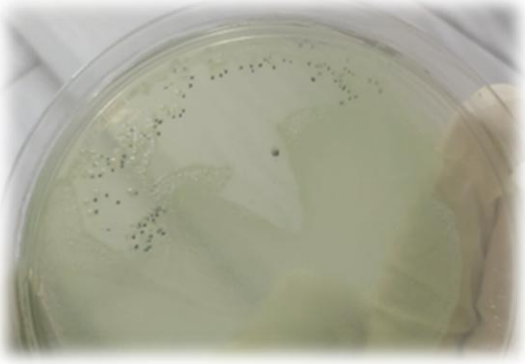


Figure (6): Recombinant NM522 genetically engineered with successful cloning on Nutrient agar contain ampicillin.

Production and Purification of recombinant thrombolytic enzyme

Production and Purification recombinant thrombolytic enzyme from recombinant NM522 clone via utilizing Ammonium sulfate, Diyalisis, Ion Exchange and Gel filtration chromatography for (8) transformants NM522 clone that express recombinant thrombolytic enzyme as shown in table (5) and figure (7).

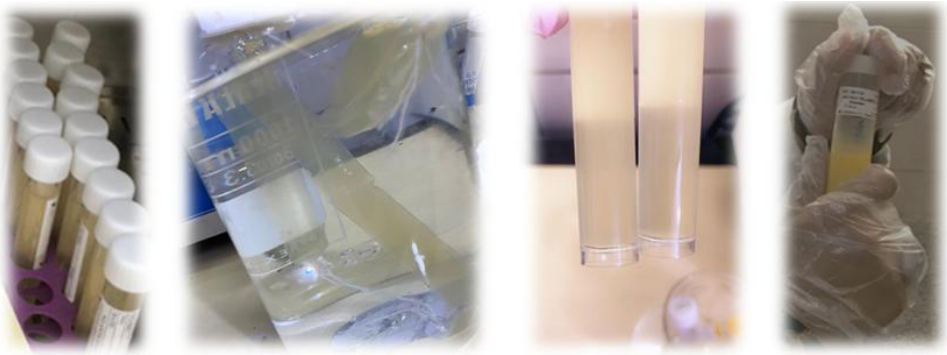
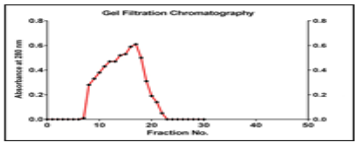


Figure (7): Purification of recombinant thrombolytic enzyme produce from NM522 clone.

Table (5): Absorbance at 280 nm with fraction number and protein concentration with peak for purification recombinant thrombolytic enzyme by Gel filtration chromatography.

frac.no.	Absorbency at 280nm	Prot. conc.
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0.01	0
9	0.28	0.09
10	0.33	0.09
11	0.38	1.03
12	0.43	1.02
13	0.47	1.02
14	0.47	1.05
15	0.52	1.06
16	0.53	1.08
17	0.59	1.06
18	0.61	1.08
19	0.5	1.08
20	0.31	1.20
21	0.19	1.21
22	0.14	1.20
23	0.05	0.06
24	0	0,05
25	0	
26	0	
27	0	
28	0	
29	0	
3·	0	



Ion exchange chromatography can be serve as one of the utmost efficacious way for protein separation and purification. This technicality is fast, quite simple and effective, this method depend on reversible binding of a protein to reverse charge of ion exchange. DEAE-Cellulose exchanger was utilized to purify streptokinase from *Bacillus lichniformis* [24].

$$\text{Conc.} = \frac{\text{OD.}}{\text{SIOP CONC.}} * \text{fraction volume}$$

Final SaK Conc. (1000µg/ml) in final volume (40 ml).

OD= 1.20

Slop conc. =0.006

Fraction Volume = 5

Medical applications of recombinant thrombolytic enzyme with recovery blood dissolve for thrombi *in vitro*

Medical applications of recombinant thrombolytic enzyme with recovery blood dissolve for thrombi *in vitro* (within tubes) that containing blood clot (thrombi) to limit activity of recombinant thrombolytic enzyme and determination of recombinant thrombolytic enzyme Activity, results shown dissolve thrombi when adding 40,50 and 100µl when purified recombinant thrombolytic enzyme within few seconds. All purified enzyme concentration 10, 20,30,40,50,100 µl has. Also *in vivo* within mice. The clot formed in the laboratory was examined for human blood and the clot was examined inside mice. The results showed that the blood was restored to its liquid state and the clot was dissolved quickly within seconds. Likewise, inside mice, the blood was dissolved within a very short period after the process of inducing the formation of the clot was carried out inside the mice and examined after injecting the enzyme inside the mice as shown in figure (8).

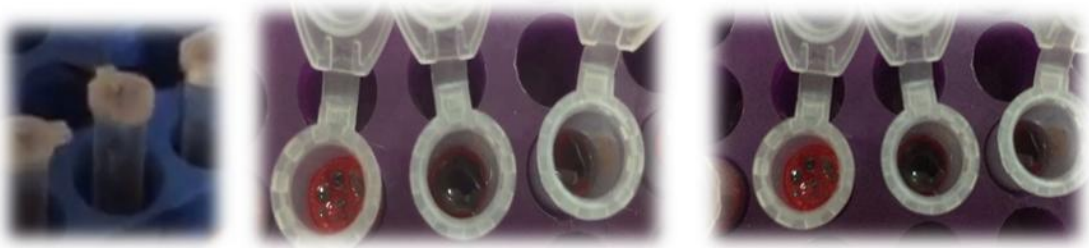


Figure (8): Medical applications of recombinant thrombolytic enzyme with recovery blood dissolve for thrombi *in vitro*

Effective evolution of recombinant DNA-derived pharmaceuticals, a modern class of curative agents, is specified by a diversity of factors simulating the selection and status of compound beneath evolution. Technical evolution of recombinant DNA technology set genetics in the mainstream of medicament. Techniques for gene transmit have been afflicted to insert synthetic sequences into the cellular genomes [25].

CONCLUSIONS:

1- The mutant bacteria transitioned from antibiotic-resistant to antibiotic-sensitive.
2- Second, the mutant bacteria transitioned from low or non-producing to producing a high level of the thrombolytic enzyme.
3- Third, the cloning process in genetic engineering successfully engineered the mutant gene from the mutant bacteria, resulting in the production of a highly effective thrombolytic enzyme for thrombosis and the treatment of blood clots and strokes.

Data Accessibility statement

The datasets analyzed and/or construed during the current treatise upon corresponding author to sensible request.

Consent to publish

All author approved the final manuscript for publish.

Founding and sponsorship

No founding was received for conducting this research.

Competing interest

The authors declare no competing interests.

REFERENCES

[1] Shaghayegh G, *et al.*, Chronic Rhinosinusitis, S. aureus Biofilm and Secreted Products, Inflammatory Responses, and Disease Severity. *Biomedicines*. 2022, 10(6):1362.

[2] Wagner, P. L. and Waldor, M. K. (2002). Study Group. *New England Journal of Medicine*, 344, 11–16.Bacteriophage control of Bacterial virulence. Infection and Immunity, 70, 3985–3993.Zasloff, M.. *Antimicrobial peptides of multicellular organisms. Nature,* 415, 389–395.

[3] Bokarewa, M.I., Jin, T., Tarkowski, A., 2006. *Staphylococcus aureus:* Staphylokinase. *Int. J. Biochem. Cell Biol.*, 38, 504– 509.

[4] Resch, G.; Francois, P.; Morisset, D.; Stojanov, M.; Bonetti, E.J.; Schrenzel, J. Sakwinska, O. and Moreillon, P.(2013).: Human-to-bovine jump of Staphylococcus aureus CC8 is associated with the loss of a beta-hemolysin converting prophage and the acquisition of a new staphylococcal cassette chromosome. *PLoS ONE*. 8: e58187-10.1371/journal.pone.0058187.

[5] Antikainen, N.M. and Martin, S.F. (2005). Altering protein specificity: techniques and applications. *Bioorganic & Medicinal Chemistry*, Vol. 13, No. 8, pp.2701- 2716, ISSN: 0968-0896.

[6] Mousumi,D.; Prasad, G.B.K.S. and Bisen, P.S.(2010). Recombinant DNA Pharmaceuticals. Molecular Diagnostics: Promises and Possibilities Dordrech Heidelberg London, Springer, pp. 32-54.

[7] Promega Corporate Bacterial Strain NM522 Guide Agilent Technologies NM522 Competent Cells Reference.

[8] Yanisch- Perron, C., Vieira, J., & Messing, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene*, 33(1), 103-119.

[9] Messing, J. (1983). New M13 vectors for cloning. *Methods in Enzymology*, 101, 20–78.

[10] Sweta , P. ; Rajpal , S. ; Kashyap , J. Y . ; Hemant , J . P . ; Girdhar , M . T . and Hatim , F. D . (2006) . Development of an in vitro model to study clot lysis activity of thrombolytic drugs. *Thrombosis Journal , (4).*

[11] Rother, J. ; Ford , G . A . and Thijs ,V. N.(2013). Thrombolytics in acute ischaemic stroke: historical perspective and future opportunities. *Cerebrovasc Dis; 35(4): 313-9.*

[12] Biomeruex corporation. (2010) . VITEK . USA .

[13] Naseer,S. B., Ravi, M., & Subhashchandra, M. G. (2014). Screening of staphylokinase producing Staphylococcus aureus from clinical samples. Int J Res Biol Sci, 4(2), 46-48.

[14] MacFaddin, J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. Lippincott Williams & Wilkins, Philadelphia.

[15] Mohammed, N. R. (2021). Biological activity of Nd:YAG laser and beta, gamma radiation on *Klebsiella pneumoniae* resistance colistin. *Systematic Reviews in Pharmacy*, 12(4).

[16] Annesley TM. Giving credit: citations and references. Clin Chem. 2011;52 (1):14-17.

[17] Christiansen S, Iverson C, Flanagin A, et al. AMA Manual of Style: A Guide for Authors and Editors. 11th ed. Oxford University Press; 2020.

[18] Kai, M., Effmert, U. & Piechulla, B. Bacterial-plant-interactions: approaches to unravel the biological function of bacterial volatiles in the rhizosphere. *Front. Microbiol.* 7, 108b (2016).

[19] Green, M. R., & Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Cold Spring Harbor Laboratory Press.

[20] Bradford, Marion (1976). "A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding" (PDF). *Analytical Biochemistry*. 72 (1–2): 248–254.

[21] Banerjee, A. ; Chisti ,Y. and Banerjee, U.C. (2004). Streptokinase - a clinically useful thrombolytic agent. *Biotechnol Advances; 22: 287-305.*

[22] Kim, J. Y., Kyung, S. Y., Park, J. W., & Choi, J. C. (2007). Comparison study of the extraction methods of paraquat in post-mortem human blood samples. Archives of Pharmacal Research. Volume 30, Issue 2. Pp. 234–240.

[23] Hwang, K. J., et al. "Purification and Characterization of a New Fibrinolytic Enzyme of *Bacillus licheniformis* KJ-31, Isolated from Korean Traditional Jeot-gal." *Journal of Microbiology and Biotechnology*, vol. 17, no. 9, 2007, pp. 1469–1476

[24] AL-Juamily,E.F. and Al-Zaidy(2012). Optimization conditions of production fibrinolytic enzyme from *Bacillus lichniformis* B4 local isolate. Br. *J.Pharmacol. Toxicol.* Vol: 3, 289-295.

[25] Singh, J., & Hussain, A. (Eds.). (2010). Molecular Diagnostics: Promises and Possibilities. Springer Netherlands. doi.org