Ann. For. Res. 66(1): 2382-2389, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

THE OPTIMUM METHOD FOR EXTRACTING AND PURIFYING COAGULASE ENZYME FROM *STAPHYLOCOCCUS AUREUS* THAT ISOLATED FROM CLINICAL SAMPLES

Noor S. Hadi , Entesar H. Ali
Division of Biotechnology, Department of Applied Sciences, University of Technology, Baghdad, Iraq, E-mail: @ nonsbo@gmail.com

ABSTRACT

Staphylococcus aureus is a Gram-positive coccus that is normally found in both human nasal passages and skin. It is a facultative anaerobic cocci. Suppurative abscesses are also caused by S. aureus.. *S. aureus* have Willebrand factor-binding protein and coagulase that causes the coagulation of blood. Coagulase enzymes can be used in future to increase the coagulation rate in people who have problems with blood clotting. Therefore, the current study aimed to determine the optimal method for extracting the enzyme from staphylococcal bacteria isolated from clinical samples. Patients between the ages of 18 and 45 had their clinical samples taken. Al-Wasiti Teaching Hospital and Al-Kindi Teaching Hospital provided samples. The research was carried out between December 2021 and March 2022. Among the 100 samples taken, 20 were from the skin, 35 from burns, 30 from wounds, and 15 were urine samples. The enzyme was isolated using four distinct techniques (crude extract, enzyme dialyzes, DEAD-cellulose, and Sepharose 6B), after which the enzyme's purification, folding, total activity, specific activity, protein condensation, and coagulase activity were assessed. The findings indicated that crude extract produced a higher yield, but at the expense of purity compared to other approaches. In contrast, Sepharose 6B methods demonstrated higher purity with lower amounts. In comparison to the first and second methods, DEAD-cellulose produced results that were in the middle, with relatively high purification factors. The volume of the product was large compared to the fourth method, and the enzyme had the highest overall activity value of all the methods used in the current study. It appears to be the most effective technique for coagulase enzyme extraction. The study came to the conclusion that each method has benefits and drawbacks, including yields in large amounts but without purity, and that it is appropriate for extracting the enzyme for purposes that require large amounts, even if they are of low purity. There are other techniques that produce high purity in smaller amounts and are appropriate for applications that call for very pure enzymes.

Keywords: Coagulase, *Staphylococcus*, Extraction, DEAD-cellulose, Sepharose 6B

1. INTRODUCTION

Staphylococcus aureus is a facultative anaerobic Gram-positive coccus and a member of the normal skin flora as well as the nasal passages of humans. S. aureus is also the etiological agent of suppurative abscesses [1], [2]. *S. aureus* is a frequent cause of bacteremia, pneumonia, skin and soft tissue infection as well as osteomyelitis and septic arthritis[2], [3]. *S. aureus* displays several striking microbiological properties, e.g., the microbe binds immunoglobulins and agglutinates with

or coagulates blood and plasma . These traits have been useful for the early and rapid diagnosis of *S. aureus* infections [4], [5]. Following entry into the bloodstream of infected hosts, *S. aureus* strains disseminate into tissues and seed abscesses . Staphylococci multiply as a bacterial community at the center of these lesions, separated from infiltrating immune cells by an amorphous pseudocapsule [6], [7].

S. aureus the Willebrand factor-binding protein and coagulase of the host organism, which aid in the teichoic acids in the bacterial cell wall adhering to the endothelium cells of the host organism. Blood coagulation is triggered by this adhesion process, which is started when prothrombin undergoes non-proteolytic conversion into the active enzyme staphylothrombin, which then interacts with fibrinogen to create fibrin threads. [8], [9]. As a result, the clot creates a visible fibrin barrier that prevents the germs from deteriorating within the host. [6], [10]. Even in the absence of calcium and in the presence of anticoagulants such sodium citrate and ethylene-diamine tetra acetate, this response was seen. As a result, the protein might be employed as a crucial agent in circumstances when blood coagulation is severely compromised. [11].
Previous study [12] referred to the interaction of enzyme and blood leads to the conversion of fibrinogen to fibrin, a process that occurs in a test tube without calcium and with anticoagulants like heparin and dicoumarol present. The administration of large intravenous doses of coagulase to rabbits may result in in vivo defibrinogenation, sequestration of fibrin in tissues such as the lungs, and even in acute death.
By activating prothrombin, S. aureus causes coagulation to occur immediately. Additionally, S. aureus controls fibrinolysis via plasminogen activation through staphylokinase.Also S. aureus interacts with vital coagulation proteins including fibrin(ogen), fibronectin, and von Willebrand factor in addition to binding and activating platelets. S. aureus obtains a considerable edge over the host defensive systems by modifying the coagulation system. Therefore, investigating how S. aureus interacts with the hemostatic system may result in the development of novel, creative treatments for blood coagulation disease. [13], [14].
One of the most important applications that can use the coagulase enzyme in the future is to increase the coagulation rate in people who have problems with blood clotting, as the enzyme has been proven in a previous study that it is not affected by anticoagulants and calcium deficiency. There is no previous study specialized in testing the effectiveness of the enzyme in the treatment of blood clotting diseases. The current study aims to determine the optimal method for extracting the enzyme from staphylococcal bacteria isolated from clinical samples.

2. MATERIALS AND METHODS

2.1. Sample collection

Clinical samples were collected from patients between the ages of 18 to 45 years. Samples were collected from Al-Wasiti Teaching Hospital, Al-Kindi Teaching Hospital. The study was

conducted from December 2021 to March 2022. One hundred samples were collected, 20 of them from the skin, 35 from burns, 30 from wounds, and 15 from urine.

2.2. Diagnosis

Diagnosis of *S. aureus*. was determined according to Bergey's manual of systematic bacteriology [15].

2.3 Detection Enzyme Activity

The plasma was dilute 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8 ml of saline). 3 small test tubes and label as T (Test) , P (Positive Control) and N (Negative Control) was taken. Test is 18-24 hour broth culture, Positive control is 18-24 hr *S. aureus* broth culture and Negative control is sterile broth. 0.5 ml of the diluted plasma was pipetted into each tube. 5 drops (0.1 ml) of the Test organisms was added to the tube labeled "T", 5 drops of *S. aureus* culture to the tube labeled "P" and 5 drops of sterile broth to the tube labeled "N". After mixing, was incubated the three tubes at 35-37 Degree Celsius. After 1 hours clotting was examine . A tube coagulase test was performed to investigate the agglutination effect of the enzyme as described by Katz in 2010 with some modification [16].

2.4. Enzyme purification

Coagulase purified according to [17], [18].

2.4.1. Enzyme Extraction

Heart infusion broth was used for coagulase production. Staphylococci were grown in heart infusion broth for 3 days at 37 C on a rotary shaker. The organisms were removed by centrifugation at 20,000 rpm in a continuous flow centrifuge.

2.4.2. Enzyme dialyzes

The crude enzyme was then dialyzed against (o.oo1) M phosphate buffer, pH (6.8) for 24 hr.

2.4.3. Ion exchange chromatography (DEAE-Cellulose)

It was prepared according to [19] as follows:

A ten milliliters sample after the dialysis step was added to the ion Exchange column. The flow rate was organized to be 60 ml/hr., and elution was Collected as 5 ml fraction. Enzyme that bound the gel was eluted by an elution buffer and Gradient NaCl in a concentration of (0.2-1M). The absorbance for each fraction was measured at 280 nm . Coagulase activity was estimated and protein concentration was done for peaks as described perversely.

2.4.4. Gel filtration chromatography (Sephadex G-100)

An active concentrated fraction sample was loaded over the Sephadex G-100. An equilibration buffer was used to collect the fractions. The flow rate was organized to be 30 ml/hr., and a fraction volume of 5 ml was collected. Absorbance at 280 nm was measured for each fraction peak. Measurement of coagulase activity and protein concentration was done.

2.5 Calculate Enzyme Activity

The enzyme activity was calculated using the following equation:

Enzyme activity =rate × reaction volume.

Specific activity was calculated by dividing the enzyme units (U) with the protein content.

Total activity was calculated using the following equation:
Total activity = enzyme activity*total step volume
Purification (fold) was calculated using the following equation:
Purification (fold) =specific activity (for steps)/ specific activity (crude enzyme).

3. RESULTS AND DISCUSSION

3.2. Evaluation Activity of Coagulase Enzyme

The enzyme was extracted by four different methods(Crude extract, Enzyme dialyzes, DEAD-cellulose, Sepharose 6B). The precipitation process with neutral salts is one of the necessary processes in purifying enzymes to get rid of unwanted proteins that are present with the enzyme. The enzyme was collected by a refrigerated centrifuge at 10,000 rpm for 15 minutes. the results showed in crude extract methods volume was 500ml, enzyme activity was 31 U/ml , protein concentration was 25 mg/ml , specific activity was 1.2 U/mg , total activity 15500 U and purification folds was 1.
For the purpose of getting rid of the ammonium sulfate salts, the purification was used by dialysis bags, where a volume of 15 milliliters of the enzyme obtained from the precipitation step was placed with ammonium sulfate and placed in the dialysis tubes. The results of the purification showed an increase in the specific activity of the enzyme up to 2.6 U/mg protein compared to the specific activity after the precipitation step with ammonium sulfate 1.2 U/mg protein,
After the sedimentation and dialysis step, 10 milliliters of the enzyme solution was passed through an ion exchange column - DEAE Cellulose, and then the absorbance was measured at a wavelength of 280 nm, the results showed specific activity up to 70 U/mg ,and total activity to 43750 U.
The gel filtration technique was the last step in enzyme purification, where the parts obtained from the ion exchange purification step were collected and placed in a gel filtration column containing Sephadex , then the absorbance was measured at a wavelength of 280 nm, and the results shown increasing in all parameters (enzyme activity was 650U/ml , protein concentration was 0.8 mg/ml , specific activity was 813 U/mg , total activity 26000 U and purification folds was 678. as shown in table 1

Table (1) Coagulase enzyme extracted by four different methods(Crude extract, Enzyme dialyzes, DEAD-cellulose, Sepharose 6B).

Purification step	Volume (ml)	coagulase activity (U/ml)	Protein conc. (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification folds
Crude extract	**500**	**31**	**25**	**1.2**	**15500**	**1**
Enzyme dialyzes	**400**	**50**	**19**	**2.6**	**20000**	**2**
DEAD-cellulose	**250**	**175**	**2.5**	**70**	**43750**	**58**
Sepharose 6B	**40**	**650**	**0.8**	**813**	**26000**	**678**

In enzyme extraction, there appears to be a relationship between some variables, enzyme purity, quantity and cost of enzyme extraction methods . The result of the Crude extract method showed large volume(yield) with high protein concentration (impurities). While Sepharose 6B method showed low yield 40 ml with higher purity , protein concentration very low 0.8 mg /ml, also higher purefection fold 678.

In a previous study [20] extracted enzyme using Crude extract found protein concentration 45 mg/ml while in current study 25 mg/ml.

Dialysis is another common laboratory technique for the purification, concentration, or fractionation of enzymes . Dialysis works on the principles of the diffusion of solutes and ultrafiltration of fluids across a semipermeable membrane or dialysis bag, which contains the carbohydrate solution [21], [22], dialyzes also showed low purification folds that is because the loss of the target protein, especially the step of buffer equilibration. The protein will bind to any surface it can contact, and shear forces, foaming, and rapid changes in ionic strength can easily inactivate the protein [23], [24].

DEAD-cellulose showed medium results, where the purification foldes were rather high compared to the first and second methods. Also, the highest total activity value of the enzyme from all the methods used in current study , and the volume of the product was large compared to the fourth method.It seems that it is the best method for extracting the enzyme coagulase.

The advantages of this method are that more than one protein can be separated and purified simultaneously, the expensive affinity column is protected from contamination by the impurities in the mouse serum, and it is fast, selective, robust, and reproducible [25].

The results showed the best fraction that showed higher enzyme activity was in 20 fraction, as shown in figure (1). That is agreed with previous study [20] that found higher value of enzyme activity in friction no. 20-50.

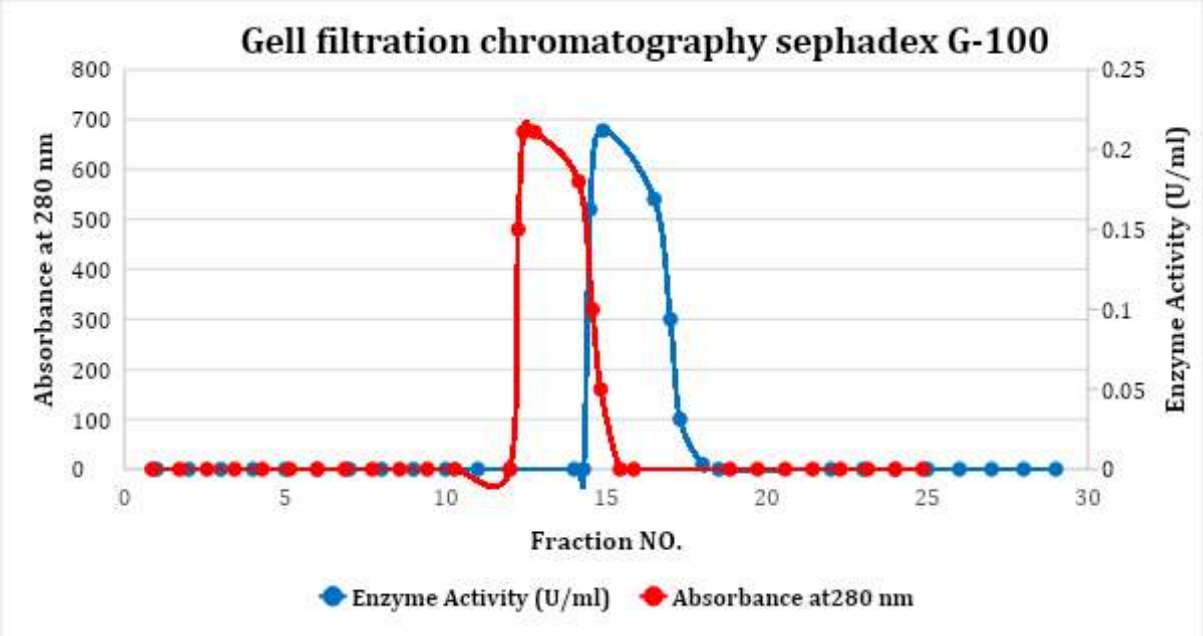

Figure (1) Extraction coagulase enzyme from *s.aureu*s using ion exchange chromatography.

Sepharose 6B showed the best results in terms of purity, selectivity and quality compared with other methods used in this study.

The results also showed the beast fraction no. have higher enzyme activity was in number 10 fraction that showed 650 U/mg of coagulase activity. as showen in figure (2)

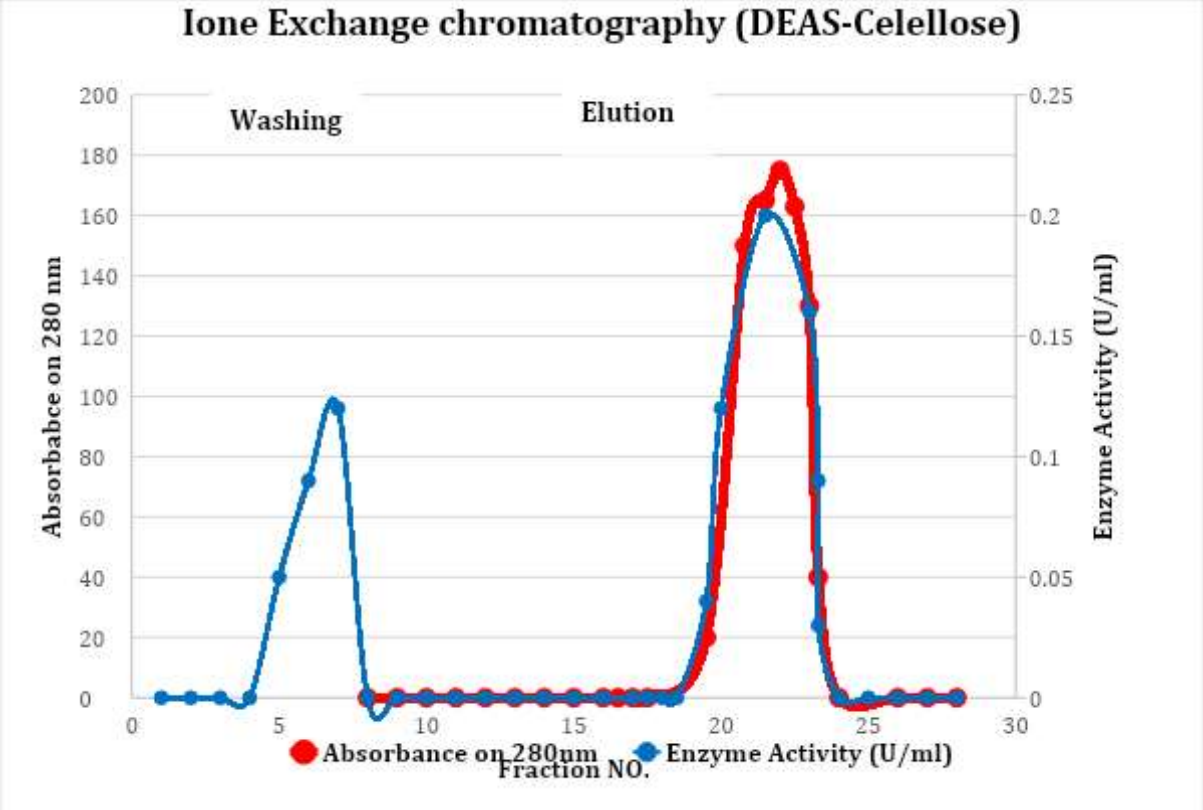

Figure (2) Evaluation coagulase activity that extracted by Sephadex chromatography methods

Conclusion

The study concluded that each method has advantages and disadvantages, including yields in large quantities, but without purity, and it is suitable for extracting the enzyme for purposes that require large quantities, even if they are of low purity. There are other methods that give high purity, but in small quantities, and they are suitable for purposes that require an enzyme of very high purity. DEAD-cellulose showed medium results, where the purification foldes were rather high compared to the first and second methods. Also, the highest total activity value of the enzyme from all the methods used in current study , and the volume of the product was large compared to the fourth method.It seems that it is the best method for extracting the enzyme coagulase.

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