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Extraction and Purification of Staphylolysin Enzyme from Local Isolate of *Pseudomonas Aeruginosa*

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Abstract :

Staphylolycin is one of the important enzymes produced by *Pseudomonas aeruginosa* as a competitive way against other bacterial species such Staphylococcus aureus.*Pseudomonas aeruginosa* was isolated from a number of clinical specimens, including urine, sputum, wound, and burn swabs. On Tryptic soya agar with heat killed *Staphylococcus aureus* at temperature 100°C, the capacity of local isolates to produce staphylolysin enzyme was investigated. The Staphylolytic activity was extracted by cooling centrifugation and partially purified by ammonium sulphate precipitation at a saturation percentage of 80%, followed by Ion exchange chromatography using a Dowex-column (at 1.5 M NaCl) with purification folds and recovery of 2.04 fold, while SEC gave 1.51 fold .

Key words : staphylolysin , *Pseudomonas aeruginosa , Purification* .

Introduction :

Pseudomonas aeruginosa is a gram-negative, aerobic rod-shaped bacterium that belongs to the Pseudomonadaceae bacterial family and is a member of the -proteobacteria [1]. One of this organism's peculiar characteristics, as well as its potential for significant tissue destruction, is its association with the synthesis and secretion of many proteolytic enzymes. These include elastase, alkaline protease, and the LasA and LasD elastolytic and staphylolytic enzymes [2]. LasA and LasD are staphylolytic enzymes that are extracellular proteolytic enzymes that are believed to be involved in the pathogenesis of this organism [3]. Consequently, a lysine-specific endopeptidase was identified, as well as an endopeptidase capable of lysing Staphylococcus aureus cells by cleaving the pentaglycine bridges within their

peptidoglycan network.[4]. Elastase is the most abundant endopeptidase. Additionally, it exhibits an exceptionally strong substrate specificity and is capable of degrading a large number of host proteins. This involves elastin, a significant constituent of connective tissues that is resistant to hydrolysis by the majority of proteases. Elastase is thus considered a major virulence factor of *P. aeruginosa*. Alkaline proteinase has a broad specificity for cleavage but is less potent than elastase and lacks elastolytic activity [5].This study aimed to isolate and purify of staphylolysin enzyme from *Pseudomonas aueroginosa.*

Materiales and methods :

Isolation and identification of *Pseudomonas aeruginosa*

All samples were collected from October 2020, it was about twenty two of *Pseudomonas aeruginosa* clinical isolates were isolated from local different clinical sources in Ramadi teaching hospital. . Under aseptic conditions in the laboratory, the specimens were streaked on blood agar and MacConkey agar and incubated for 24 hours at 37 ° C[6] [7] [8] .

Gram stain ability , growth at 42ºC according to [6] and routin biochemical tests were examined for all bacterial isolates . Identification of bacteria using automated VITEK-2 device (bioMérieux, France) methods using ID-GNB cards has been checked according to the manufacturer's instructions.

Qualitative test of staphylolysin production :

The ability of isolates to produce the staphylolysin enzyme was tested by using Trypticase soya agar with 0.2% (w / v) of killed S. aureus bacteria by 100 ℃ , then pseudodomonas was streaked on TSA+0.2 S.A and incubateed two day at 37℃ [9].

Quantitative staphylolysin production from :

For the purpose of quantitatively producing staphylolycin, in th first the sample under study was activated on a TSB medium for 24 hours, then a ratio of 1% was taken from activated bacteria in a 300 ml TSA volumetric flask and placed in a shaker incubator for 48 hours, 180rpm/min and 37 ℃ [9] , then cooling centfugation at 12000 rpm/min for 13 min . finally , Supernatant was taken which represents crude enzyme .The

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staphylocin A and D enzymes were estimated according to [10] , while protein concentration was estimated by Bradford method[11].

Estimation of Staphylolytic enzyme assay.

Staphylolysin activity was determined by measuring the capacity of the boiled *Staphylococcus aureus* cells of *P. aeruginosa* culture surnatants for lysis. A 30ml volume of an overnight *S. aureus* culture was boiled for 10 minutes and then centrifuged at 5000 rpm for 10 minutes. The pellet was then resuspended in a solution of 10 mM Na2PO4 (pH 7.5) to an OD600 of approximately 0.8. A 100 µl aliquot of bacterial supernatant was then applied to 900 µl suspension of S. *aureus*. and after one minute, the OD600 was calculated[10].Purification of the staphylolycin enzyme: The technology process was carried out according to the following steps:

1- Ammonium Sulphate precipitation :

Ammonium sulfate crystals were added to the enzymatic extract gradually to obtain appropriate (20 , 40 , 60 ,80%) saturation in ice bath with constant stirring, then leave the solution at a temperature of 4 degrees with constant stirring. The solution was centrifuged at 12000 rpm for 30 minutes at 4 ° C [10].

2- Dialysis

The dialysis process of the two enzymes was carried out after precipitation with ammonium sulfate using dialysis bag with cut off 10-KDa and Tris buffer - HCl (0.02 M and pH = (7.5), at 4 C for 24 hours, with constant stirring.

3- Ion exchange chromatography

This step was done with Dowex -400 ion-exchanger column that was previously washed and equilibrated with a Tris - Hcl (0.02 mL) and pH = 7.5 at a flow rate of 2 mL/ Minute . Enzyme was eluted with 2 M of NaCl gradient buffer Tris - Hcl (0.02 mL) and pH = 7.5 All enzyme purification steps were performed in in cooled conditions, the protein concentration and the enzyme's activity were estimated [12].

4- Size exclusion chromatography :

Further purification step was achieved using Syphacryle S-300 column (90× 2.6) to get homogenous protein. Flow rate 3ml / min.

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Results and discusion :

The phenotypic results of the detection of trypticase soya agar medium showed that there is a variation in the production of the enzyme staphylolysin, and this is due to the source of the isolation, as well as the strength of the gene encoding this enzyme figure 1.

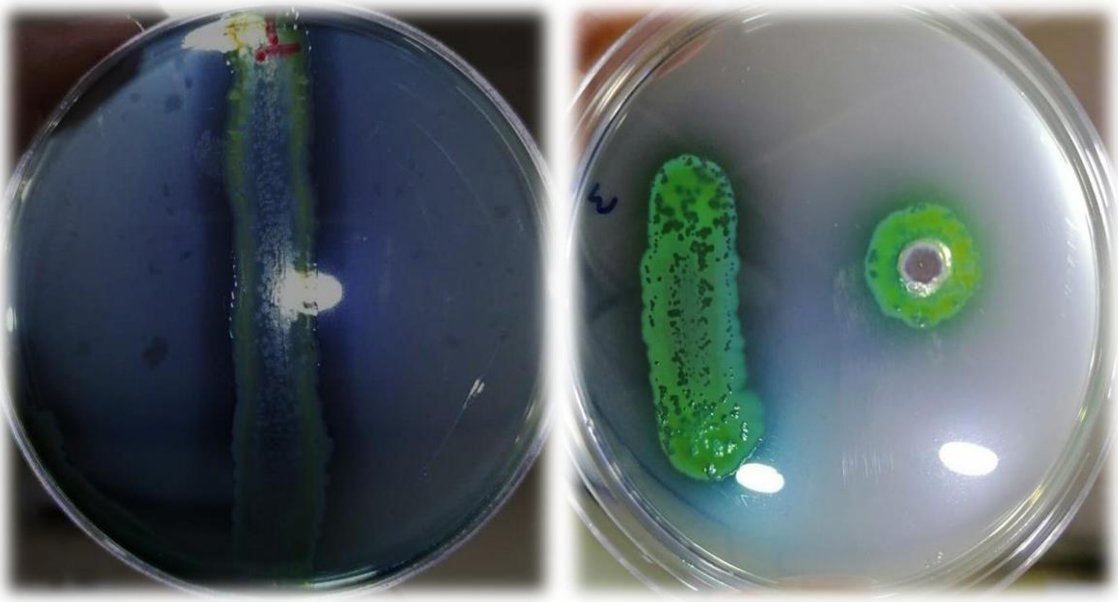

Figure 1 : Staphylolysin enzyme production on trypticase soya agar.

The isolation and purification of *P. aeruginosa* staphylolysin required the following steps: cold centrifugation of media containing the extracellular enzyme to obtain the crud extract enzyme, which was then precipitated with various concentrations of ammonium sulfate , then dialysis, Dowex 1X2-400 anion exchange column chromatography, and gel filtration chromatography using **Sephacryl S-300 table 1** .

Fractination with ammonium sulphate

The enzyme activity in different concentrations of ammonium sulfate is depicted in Figure 2, with staphylolysin exhibiting the highest specific activity at a fraction of 80% with a specific activity of 1.53 u/mg.

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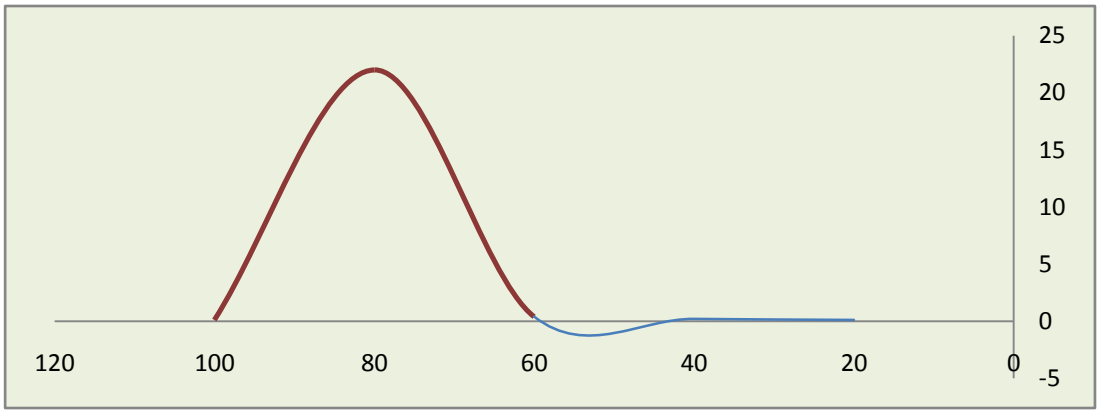

Figure 2 : Fractionation of staphylolysin preciptation .

Dialysis :

The sedimentation step followed with ammonium sulphate, Tris-Hcl dialysis process 0.02 M, pH (7.5)

Ion exchange chromatography

According to figure 3 , Purification of the enzyme using the Dowex-400 column began with establishing a buffer pH appropriate for IEC column. The experiment revealed that the enzyme was incapable of changing the counter ion between pH 5.0 and 8.8 . Figure 2 illustrates the protein pattern (A280) and activity (u/mL) of the enzyme as determined by Dowex-400 column chromatography. The activity of staphylolysin was 47.96, the purification folds were 2.04, and the enzymatic yield was 6.97 percent, as shown in this figure. These findings corroborate the researchers' findings [12][13][4].

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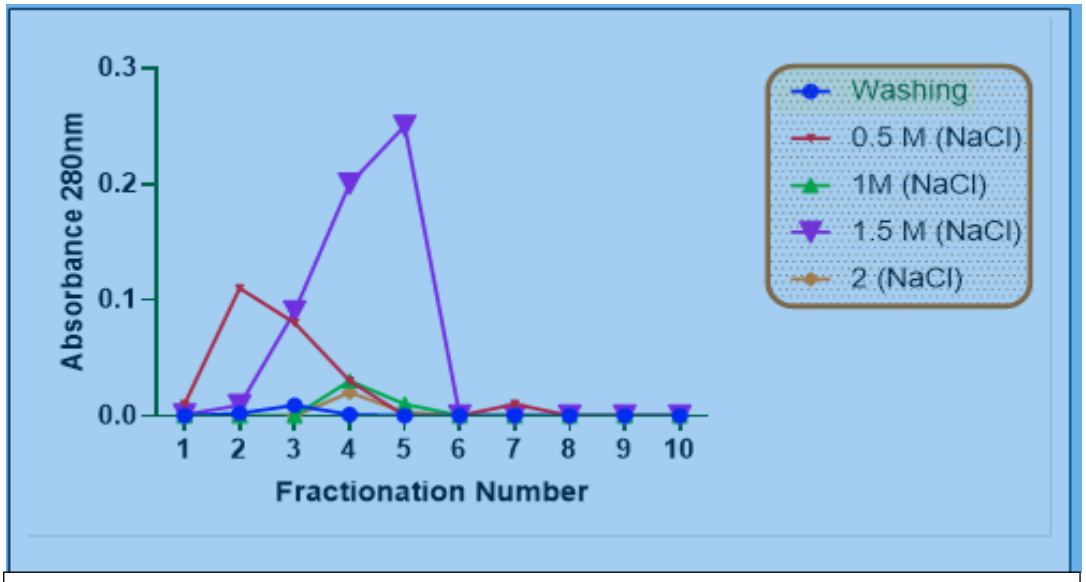

Figure 3 : Chromatogram of staphylolysin from ***Pseudomonas aeruginosa*** IEC column. Staphylolysin obtained from IEC column 20 mL;; elution solution of NaCl 0.5-2 M, pH 7.5; column size 20 mL; flow rate 1 mL/min.

Size exclusion chromotography using Sephacryl-300

According to 4 , Purification of the enzyme using gel filtration chromatography with Sephacryl S-300 employed an elution buffer of Tris-HCl 0.02 M, pH 7.5. The protein pattern (A280) and activity of staphylolysin as calculated by Sephacryl S-300 column chromatography are shown in Figure 4. This figure demonstrated that only one of the four protein peaks obtained exhibited saphylolysin activity, which was fraction 25th to 29th with a ratio of 67.5 u/mg.

Table 1 : purification Staphylolysin enzyme steps

Purification steps	size	Enzyme activity unit/ml	Protien con.mg/ml	Speciefic activity	Total activity	Yeilds	Purification fold
Crude	281	12.23	9.2	1.32	3436.63	100	1
precipitation	70	22	14.3	1.53	1540	44.81	1.15
Dialysis	6	34.7	16.6	2.09	208.2	13.5	1.36
Ion exchange – Dowex 1X2-400	5	47.96	11.2	4.28	239.8	6.97	2.04
Gel filtration-Sephacryl	5	67.5	10.4	6.49	337.5	9.82	1.51

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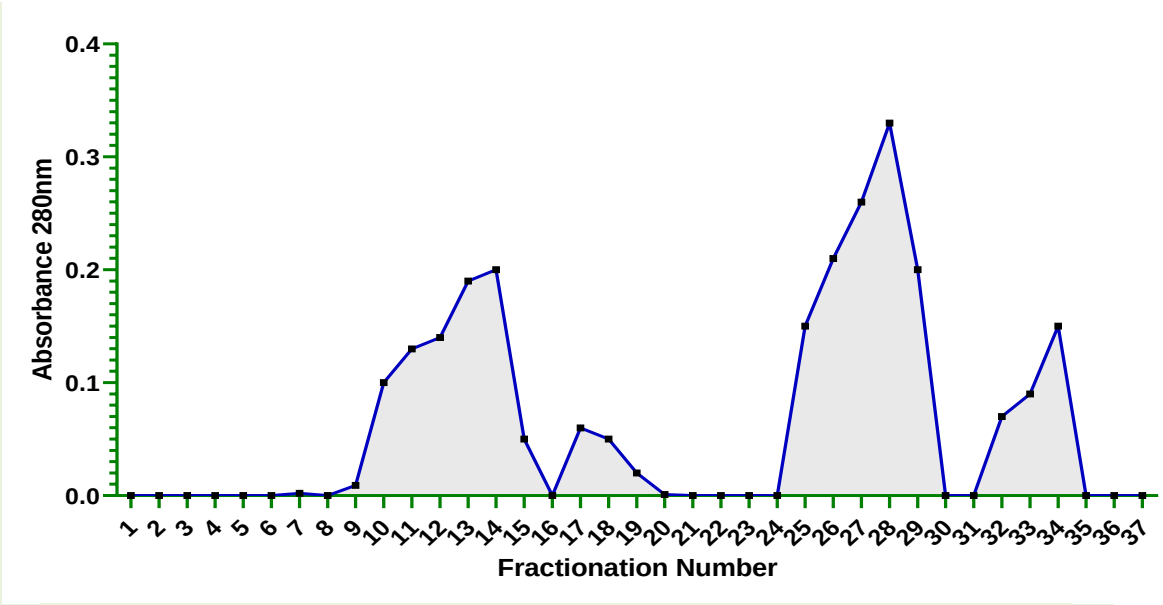

Figure 4 : Chromatogram of staphylolysin from *P. aeruginosa* at molecular sieves column chromatography using Sephacryl S-300.

Staphylolysin is a secreted metalloendopeptidase that can lyse Staphylococcus aureus cells by cleaving their peptidoglycan's pentaglycine bridges. Additionally, it is capable of degrading elastin and inducing the shedding of cell-surface proteoglycans, both of which are involved in the pathogenesis of P. aeruginosa infections. Staphylolysin is a staphylolytic endopeptidase with a molecular weight of 20 kDa that is secreted by Pseudomonas aeruginosa[14]. LasA protease has been shown to lyse a wide variety of S. aureus strains and to inhibit the growth of S. aureus cells in vitro. This indicates that LasA protease could be another useful agent in the treatment of S. aureus infections using enzymes. In experimental P. aeruginosa keratitis, LasA protease is not an important virulence factor[15].

Conclusion :

In conclusion, an important antibacterial treatment that can be used to control serious MSSA or MRSA keratitis can be provided by staphylolysin enzyme. Studies evaluating its efficacy against a variety of MRSA are intended to further develop its therapeutic potential.

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