Lipase from *S. aureus*, Purification and Application of Three Characterizing Experiments

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ABSTRACT

Many lipases have been extensively purified and characterized in terms of their activity and stability profiles relative to pH, temperature, and their effects as metal ions and chelating agents. One hundred samples were collected from different body sites and lesions of in and out patients who attented Al-Kadhumiyah and Baghdad Teaching Hospitals. Identification of bacterial isolates was performed by following the procedures mentioned elsewhere in the literature. Purification steps of lipase included: extraction of the enzyme, precipitation of the enzyme by ammonium sulphate, dialysis, ion-exchange chromatography, and gel filtration chromatography. The results revealed that the enzyme was purified with a yield of 0.3 and a fold of purification of 153.7. Also, the optimum temperature and pH for lipase stability were 40 ^{0}C and pH 8, respectively, while the molecular weight of the enzyme was 110000 daltons.

أنزيم اللايبيز المعزول من بكتريا المكورات العنقوديه الذهبيه, تنقية وتطبيق ثلاثة تجارب توصيفيه للانزيم

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الكلمات المفتاحية :المكورات الذهبية العنقودية ، اللايبيز ، ثبات الانزيم

الخلاصة

تم تنقية العديد من اللايبيزات وتوصيفها وخاصة فيما يتعلق بفعالية هذه الانزيمات وثباتيتها في ضروف مختلفه من درجات الحموضه والحراره وكذلك تاثير بعض العناصر المعدنيه والعوامل الكلابيه . تم جمع مئه عينه من من اماكن وافات مختلفه من جسم الانسان للمرضى من كلا الجنسين الذين زاروا العياده الخارجيه او كانوا راقدين في مستشفيي الكاظميه وبغداد التعليميين للفتره من اذار لسنة 2012 ولغاية ايلول من نفس السنه. وكان الغرض من جمع هذه العينات هو لعزل وتشخيص بكتريا المكورات العنقوديه الذهبيه. تم تشخيص العزلات الاولي بالطرق الكيموحيويه وبالاعتماد على الطرق القياسيه المذكوره في المصادر. تم بعد ذلك الشروع في تنقية الانزيم من خلال استخدام الترسيب بكبريتات الامونيوم والمبادل الايوني والتنقيه بالترشيح الهلامي. بينت نتائج الدراسه الحاليه ان حاصل التنقيه كان 0.3 وعدد مرات التنقيه كان 153.7. واظهرت نتائج الدراسه ان درجة الحراره المثلى لثبات الانزيم كانت 40 م في حين ان الرقم الهيدروجيني الامثل لثبات الانزيم كان 8 كما ان الوزن الجزيئي للانزيم كان 110000 دالتون.

1. Introduction

Lipases are glycerol ester hydrolases (EC: 3.1.1.3), which hydrolyze ester linkages of glycerides at water-oil interface. [1] During hydrolysis lipases pick acyl group from glycerides forming lipase-acyl complex, which then transfers its acyl group to OH group of water. [2] However, in non-aqueous conditions, these naturally hydrolytic enzymes can transfer acyl groups of carboxylic acids to nucleophiles other than water. [3]

Microbial lipases have already established their vast potential in respect to their usage in different industries. [4] In the last decades, the interest in microbial lipase production has increased [5], because of its large potential in industrial applications as additives for foods (flavor modification), fine chemicals (synthesis of esters), waste water treatment (decomposition and removal of oil substances), cosmetics (removal of lipids), pharmaceuticals (digestion of oil and fats in foods), leather (removal of lipids from animal skins) and medicine (blood triglyceride assay). [6]

Many lipases have been extensively purified and characterized in terms of their activity and stability profiles relative to pH, temperature, and their effects as metal ions and chelating agents. In many cases, lipases have been purified to homogeneity and crystallized. Generally, Purification methods have been used depending on nonspecific techniques such ammonium sulfate precipitation, hydrophobic interaction chromatography, gel filtration, and ion exchange chromatography. Chromatograph affinity has been used in some cases to reduce the number of individual needed purification steps. [7]

The study aimed to purify and characterize lipase produced by a local isolate of *Staphylococcus aureus.*

2. Materials and Methods

One hundred samples were collected from different body sites and lesions of in and out patients from both sexes who attended Al-Kadhimyia Teaching Hospital and medical laboratories in Baghdad Teaching Hospital during the period from March, 2012 until September, 2012 for the isolation and identification of *Staphylococcus aureus*. Isolated bacteria were identified biochemically depending on Holt *et al*., [8] and according to the methods described by MacFaddin. [9] Protein concentration was estimated according to the method of Bradford. [10] The method described by Bier [11] was used for the measurement of lipase activity. Lipase was purified using ammonium sulphate precipitation [12], ion exchange chromatography (DEAE-Cellulose) [13], and gel filtration chromatography (Sephacryl S - 200). [14] The optimum temperature and pH for lipase stability and the determination of its molecular weight were performed as a partial characterization steps for the purified enzyme.

3. Results

From a total of one hundred cases specimens enrolled in the current study, *S. aureus* constituted 50 % of all specimens and it was isolated as a common pathogen that can cause serious infections in various body sites and tissues (Table 1).

Table 1**:** The ranking of *Staphylococcus aureus* and the percentage of each infection from a total of 50 cases.

Cases	No. of isolates	Percentage
Wounds swabs	15	30%
Ear swabs	5	10%
Sputum	10	20%
Urine samples	6	12%
Urethral discharge	4	8%
Vaginal swabs	3	6%
Blood samples	7	14%
Total	50	100%

Lipase activity was assayed for all the fifty isolates enrolled in the current study and it appeared that isolate number 12 showed the maximum enzyme activity (600 µmole/ml) as compared to all other isolates (Figure 1).

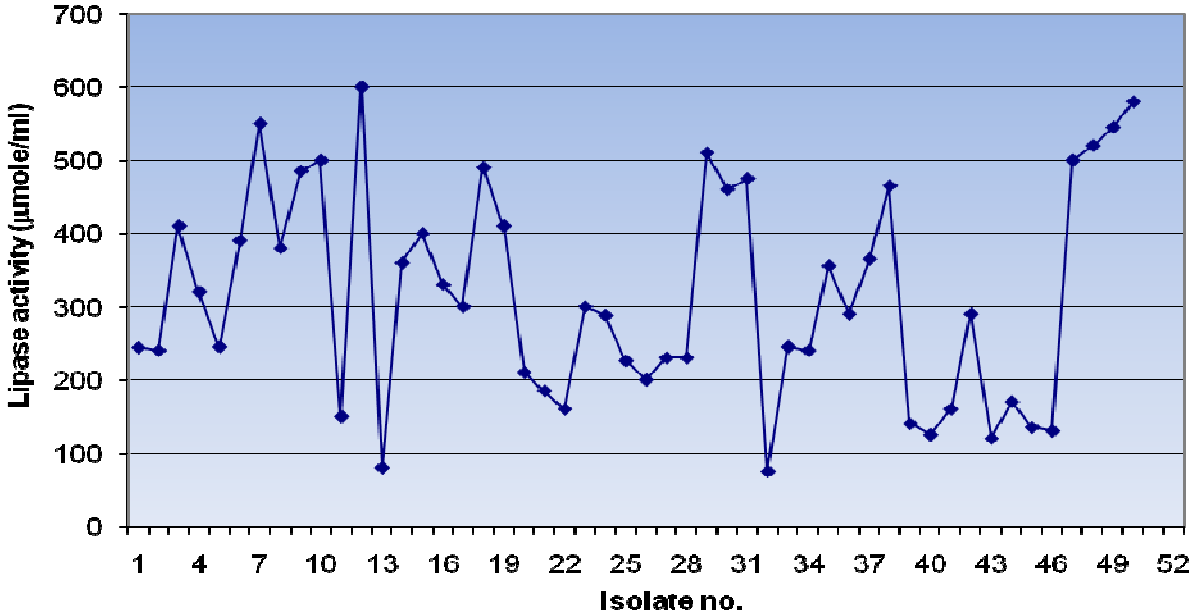

Figure 1: Isolates of *S. aureus* with different lipase activities indicated by micromole per milliliter of enzyme solution.

1) Extraction and purification of lipase.

I. Crude enzyme.

The results revealed that the crude enzyme solution had a total protein concentration of 21.3 mg/ml and an enzyme activity of 257 µmole/ ml.

II. Ammonium sulphate fractionation.

The lipase was precipitated by ammonium sulphate with 50-75% saturation percentage as a first step of purification and the results of this step revealed that the protein concentration was 4.7 mg/ml while the enzyme activity was 812 µmole/ ml.

III. Dialysis.

Lipase was dialyzed overnight against 8 volumes of 0.1M PBS with pH 7 at 4ºC. The results indicated nearly the same value of protein concentration and the specific activity of the enzyme as of the previous step.

IV. Ionic exchange chromatography by using DEAE-Cellulose.

The results showed that a prominent peak of protein resulted and it was around fraction number of 20-30 where it characterized by a maximal enzyme activity as shown in Figure 2. Protein concentration was 2.3 mg/ml and enzyme activity was 1020 µmole/ml.

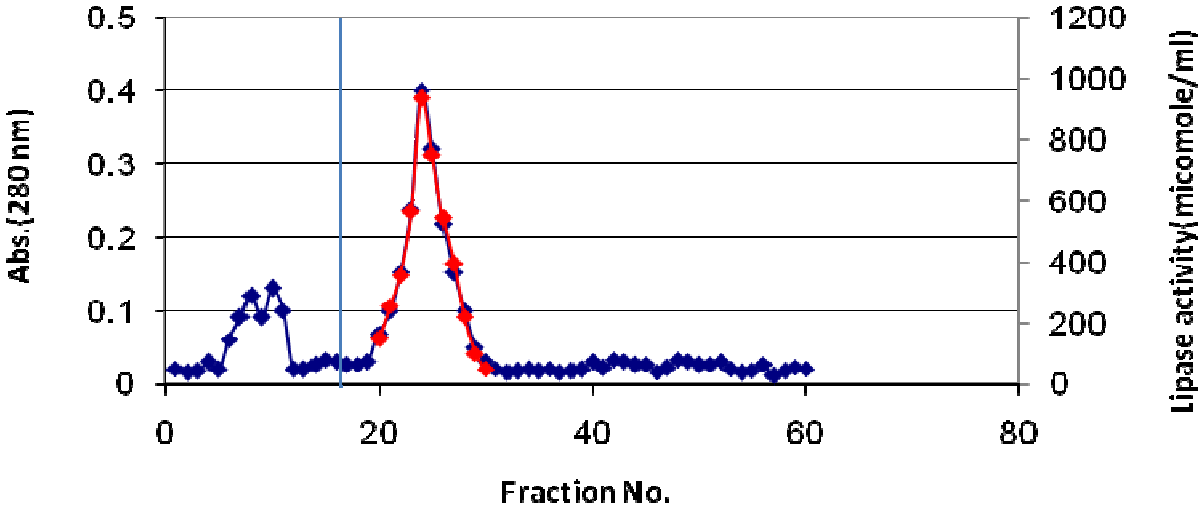

Figure 2. Lipase Ion exchange chromatography through DEAE-Cellulose column (3×8) cm. The column was calibrated with phosphate buffer saline PBS 0.1 M and pH 7.0, flow rate 40 ml/hour and 5ml / fraction.

V. Gel filtration chromatography using Sephacryl S-200 column.

Figure 3 demonstrated that single peak of protein was observed with a concentration of 0.9 mg/ml and the fractions number 31 through 41 correlated with that peak which were collected and showed an enzyme specific activity of 1669 µmole/ml.

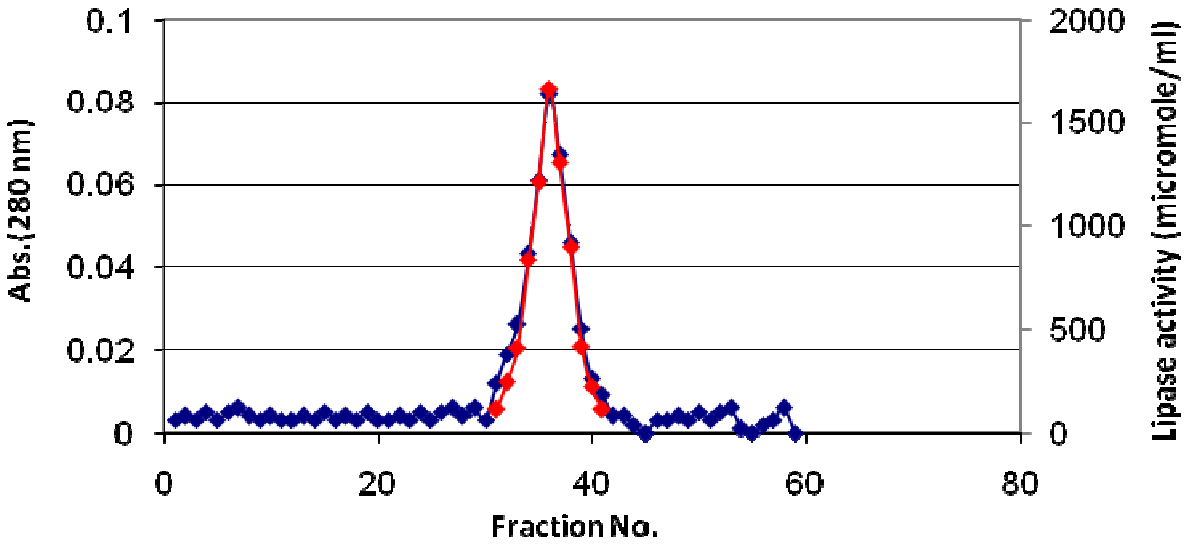

Figure 3. Purification of lipase by gel filtration chromatography using Sephacryl S-200 column (2x50) cm. The column calibrated with phosphate buffer saline PBS 0.1 M and pH 7.0, flow rate 60 ml /hour and 5ml / fraction.

Table1. Purification steps of lipase from local isolate of *Staphylococcus aureus.*

Purification step	Volume (ml)	Activity (µmole/ml)	Protein (mg/ml)	Specific activity(U/mg)	Total activity(U)	Yield(%)	Purification fold
Crude extract	100	257	21.3	12.06	25700	100	**1**
Ammonium sulphate precipitation (50-75%)	50	812	4.7	172.8	40600	1.6	**14.3**
Ion exchange using DEAE-cellulose	10	1020	2.3	443.5	10200	0.4	**36.8**
Gel filtration using Sephacryl S-200	**5**	**1669**	**0.9**	**1854**	**8345**	**0.3**	**153.7**

2) Characterization of the purified lipase

i. Determination of optimum pH for lipase stability.

The result revealed that the activity had increased with increasing pH until pH 8; afterwards lipase activity had declined steadily (Figure 4).

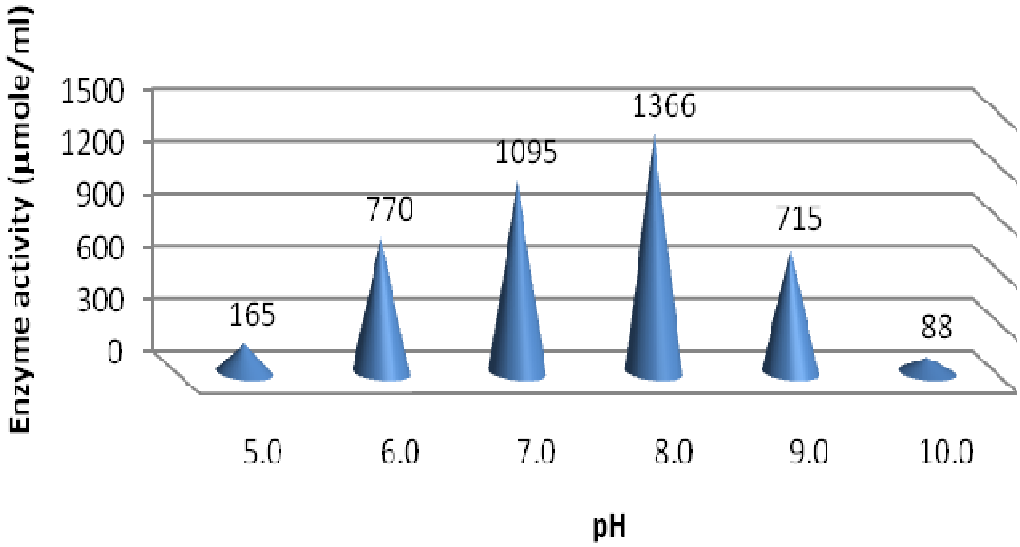

Figure 4: Effect of different pH on lipase stability.

ii. Determination of optimum temperature for lipase stability.

The result showed that lipase activity was stable as far as the temperature of incubation approached the forties Celsius but when the temperature increased, lipase activity decreased significantly (Figure 5).

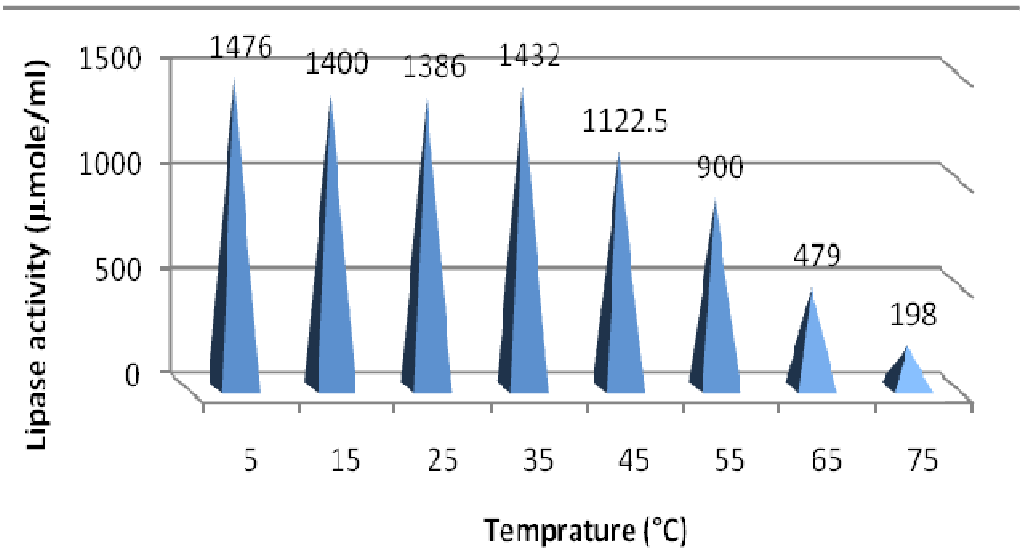

Figure 5: Effect of different temperatures of incubation on lipase activity.

iii. Determination of the molecular weight of lipase using gel filtration chromotography (Sephacryl S-200).

The construction of standard curve resembling the v_e/v_o values for the standard proteins against their relevant log molecular weight directed the way to determine the molecular weight of lipase which was about 110000 Dalton as shown in Figure 6.

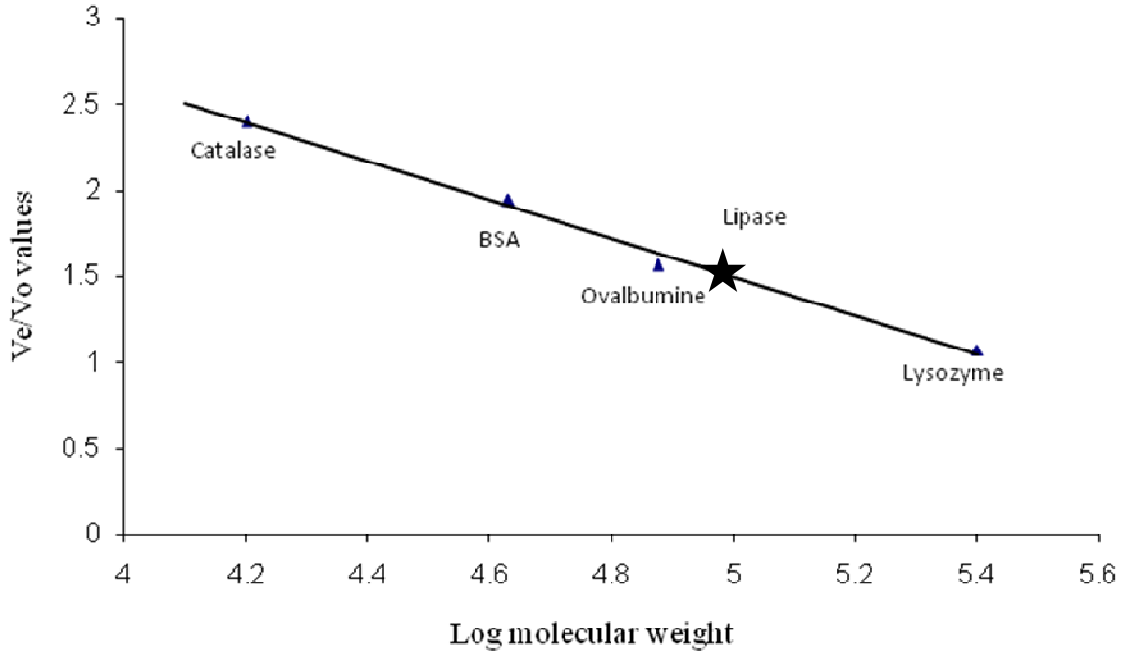

Figure 6: Determination of the molecular weight of lipase.

4. Discussion

In a previous study, lipase of bacillus was purified 2.5 fold with 24% yield by a single step of sephadex with a total protein of 134.2 mg and a total activity of 3045 □mol/ml. [15] On the other hand, the result of another study revealed that the crude extract had a protein concentration of 25.1 mg/ml and a total lipase activity of 877.5 □mol / ml. [16] In a marked disagreement with the results of the present study, it was found that the crude extract had a protein concentration of 67.44 mg and the lipase activity 37 unit/ml. [17]

Lipase is concentrated during early steps of purification to get rid of high percentage of water and to achieve an acceptable purity. Ammonium and sodium salts are among the most widely used salts for this purpose. Precipitation by salts depends on neutralization of protein surface charges and disintegration of water shell surrounding the proteins; this will eventually lead to decrease protein solubility culminating in its precipitation. Ammonium salts are mostly used for protein precipitation due to their high water solubility, availability, low cost, and non denaturing nature for the protein of interest. [18]

Massadeh *et al.*, [19] reported values far from those obtained in the current study; they mentioned that the purified lipase from *Bacillus Sterothermophilus* was precipitated by ammonium sulphate with 60% saturation with protein concentration of 1.92 mg/ml with enzyme activity 6.506 mol/ml. With a saturation percentage exceeding that achieved in the present study, lipase was precipitated by ammonium sulphate with 85% saturation with total protein concentration of 14.5 mg/ml and enzyme activity of 5.4 unit/ ml. [20]

In the current study, dialysis exerted no net effect on lipase activity. It's documented that dialysis is carried out to remove the traces of ammonium sulphate and had no prominent effects on both protein concentrations and enzyme activity

Ion exchange chromatography is characterized by high resolution, high capacity, easy to prepare, and the ability to reuse for several times to achieve higher degree of purity. [21] As for the current study; ion exchange and gel filtration chromatography were applied to purify lipase from *P. putida*. The activity of the purified enzyme was inhibited by mercury ions, SDS, and calcium ions while taurocholic acid stimulated enzyme activity. [22]

Also, in the current study, Sephacryl S-200 column was used to complete the purification steps with gel filtration chromatography. This gel has many characteristics including resistance to compression, rapid flow rate, easy to prepare, and long term stability that make it suitable candidate for excellent purification process. It can also be used for the estimation of the molecular weight of proteins irrespective for the charge carried by the protein and it can also be used for several times for the separation of protein. [23]

It was proposed that multiple patterns of lipases could result from change in gene expression, variable percentage of covalently linked carbohydrates, partial proteolysis and posttranscriptional modifications. [24]

The results of the current study contradict the idea that lipases from *S. aureus* and *S. epidermidis* are found highly active with optimum pH around 6, whereas they are most stable at the pH range 5-10. [25] Generally, bacterial lipase has neutral or alkaline pH value and show activity in abroad pH range (4-11).

In an agreement with the results of the current study, it was stated that maximal thermo stability of the lipase was observed in the temperature range of 25 to 35 °C. The enzyme was found to be completely stable at 30 °C after 1 h of incubation. [26]

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