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Purification and Characterization of Lipase produced by *Pseudomonas aeruginosa* isolated from some soils of Basrah Governorate

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Abstract:

The present study aimed to Purification and characterization of lipase produced by the isolate *Pseudomonas aeruginosa*. The results showed that crude lipase was purified by using the precipitation step of several concentrations ratios of cold acetone. The concentration ratio 4:1 gave the best specific activity 6 U/mg of protein. The subsequent purification step using gel filtration was showed that specific activity reached to 40.5U/mg of protein. The gel electrophoresis (SDS-PAGE) of lipase purity were determined. the result showed there is an appearance of a single protein band predominant in all purification steps, While The molecular weight of purified lipase was 68KDa

Key word: *Pseudomonas aeruginosa*, lipase extraction.

Introduction

The increasing of the industrial demand for lipases has led to the detection of new sources of enzymes with unique properties [1]. Lipases are the most important enzymes in different fields of life, they occupy a prominent situation among biocatalysts and have a wide spectrum of biotechnological applications [2; 3]. Lipases are group of enzymes which catalyze hydrolysis the carboxyl ester bonds of triacylglycerols (fats and oils) to monoglycerides, diglycerides, glycerol and free fatty acids, at the oil-water interface between the insoluble substrate phase and the aqueous phase, the location where enzyme is dissolved. In addition, lipases also catalyze synthesis reactions include esterification, transesterification and others, so constitute as one of the most important groups of biocatalysts for biotechnological applications [4; 5]. Although lipases production commercially by a large group of organisms, including plants, animals and microorganisms, but microbial lipases are occupy a special significance due to the stability and a greater variety of catalytic activities [6; 7; 8; 3; 9]. The microorganisms or their enzymes are used in a broad variety of biotechnological activities such as synthesis of biopolymers, vitamins, decontamination of soils, in the food, paper or textile industry and others activities [10; 11; 12; 13; 14]. The present study aimed to Purification and characterization of produced lipase by the selected isolate *P. aeruginosa* isolated from some soils (Oily soils) in Basra Governorate.

Methods

1. lipase producing isolate

The production of lipase in the present study was carried out by using the selected isolate *Pseudomonas aeruginosa* and the optimum conditions for lipase production according of [15], the basal salt medium for lipase production was prepared according to [16]. The medium was inoculated with 6 ml of the overnight culture was prepared according to [17].

2. Lipase crude extraction produced by *P. aeruginosa* isolate

The lipase crude was extracted according to [16]. The supernatant was collected and sterilized using 0.22µm Millipore filter paper. The filtrate collected to obtain a crude lipase in sterilize vial.

3. Lipase assay

3.1. Determination of protein content

The protein content of produced enzyme was evaluated photo-metrically by Bradford method according to [18].

3..2. Determination of lipase activity

Lipase activity was measured by titrimetric method according to the [19]. Lipase activity was calculated as micro moles of free fatty acids formed from hydrolysis of olive oil per ml of crude lipase as per equation.

$$\textbf{Activity} = \frac{(\mathbf{Vs}-\mathbf{VB}).\mathbf{N}.\mathbf{1000}}{\mathbf{S}}$$

Vs: The volume of 0.1M NaOH solution consumed by the enzyme-substrate cocktail (ml).
V_B: The volume of 0.1M NaOH solution consumed in the titration by the substrate (Control) cocktail (ml).
N: The molar strength of the NaOH solution used for titration (0.1M).
S: The substrate volume of cocktail solution (10ml). The one unit (U) of lipase enzyme is definite as the amount of enzyme, which is required to liberate (1µmol) of fatty acids from triglycerides.

4. Purification of produced lipase by *P. aeruginosa* isolate

An extracellular lipase produced by *P. aeruginosa* isolate was purified by employed a two-step procedure, chemical precipitation and gel exclusion chromatography.

4.1. Precipitation of produced lipase

The partial purification of lipase was carried out by acetone precipitation method according to protocol as mentioned [20], different ratios of acetone were used (1:1, 2:1, 3:1, 4:1, 5:1). the precipitated protein samples was stored at 4℃ for use in the next purification steps.

4.2. Dialysis (desalting) of produced lipase

fifteen ml of precipitate protein sample was poured in the dialysis sacs and was incubated in 500ml beaker containing buffer of 20 mM TrisHCl. and stored at 4℃ for (24- 48) hs and after every 6 hrs the buffer was changed [21].

4.3. Size exclusion Chromatography of produced lipase

The acetone- precipitated protein was purified by gel filtration column chromatography according to [22]. The Sephadex G-150 column resin was prepared according to the instructions of the manufacturing company.

5. Characterization of purified Lipase

Determination of the purity and molecular weight purified lipase

The SDS-PAGE (sodium dodecyl sulphate Slab Polyacrylamide gel electrophoresis) was applied on samples and their proteins resolved according to their molecular weight differences according to [23]. The analysis of gel electrophoresis and molecular weight calculation of lipase in presence the standard protein marker by using SDS-PAGE was determinted computerized by the CS analyzer- atto program.

Results and Discussion

1. Crude Lipase production

The results of the present study showed that the crude enzyme solution had an enzyme activity about 375U/ml and protein content about 3.6mg/ml.

The results indicated that the lipase activity of crude enzyme was increased steadily with cultivation time and the best enzyme production was reached after 48hrs of cultivation. Significant lipase production was observed during the early phase of growth and then the activity was declined after increasing the incubation period, this may be due to the reality that the production of lipase has been frequently inhibited by the end products as glycerol and fatty acids.

The major factor for the expression of lipase activity always has been the carbon source, since lipases are inducible enzymes. and are thus generally produced in the presence of a lipid source such as oil or any other inducer, such as triacylglycerols, fatty acids, Tweens, bile salts hydrolysable esters, and glycerol. Among the different carbon sources used, olive oil was found to be the most suitable source. also the presence of the olive oil and peptone in the fermentation medium was found to be the most suitable substrate for maximum lipase production [24] .

2. Precipitation of produced lipase

The results of this step showed that the use of acetone concentration at ratio 4:1 gave maximum range of precipitation over other ratios of acetone concentrations (figure 1). This step of purification gave specific activity about 6U/mg of protein. Acetone precipitation allows protein in a sample that consists of undesirable components to be precipitated out. The present study suggest that an ratio 4:1 of cool acetone is active to precipitate of enzyme may be due to reduction of the power of water activity of enzymatic solution and which led to the solubility of protein in solution and then the precipitate the protein. also the present study showed to increase the specific activity of enzyme in this step may be due to the increasing of the purity of lipase by removing the undesirable substances [24; 25; 20].

3. Dialysis (desalting) of produced lipase

The results of the dialysis step showed the same values of protein content and the lipase activity to these values in the precipitation step.

4. Gel filtration chromatography of produced lipase

The results showed the presence of prominent protein peak characterized by a maximal lipase activity as shown in the figure (2), It was the accordance of activity peak with the prominent of protein peak. The specific activity about 40.5U/mg.

The conditions used in the performance of gel filtration technique including the media used of gel filtration (Sephadex G-150), the flow rate at 0.1ml/min and the column specifications which is used to separate, all these conditions involved in obtaining a high degree of purity when compared to the condition used in the previous studies.

The gel filtration technique affected by many factors, should be considered when designing a gel filtration systems these include: matrix choice, sample size and concentration, column parameters, choice of eluent, effect of flow rate, column cleaning and storage. The results in the present study agree with the study of [26; 27; 25; 24].

5. Determination of lipase purity

The analysis of the polyacrylamide gel electrophores (SDS-PAGA) (figure 3) showed the presence of predominant single band separated in the purified enzyme line that band has been absorved in all lines include the crude extract lipase, precipitated lipase and gel filtration chromatography product. The results explained on the basis that the *P aeruginosa* isolate in the present study produced an extracellular lipase during the growth in the medium containing 1% olive oil was selected as lipase production medium and this medium distinctive in their component of some minerals, therefore the strain was exploited to produce lipase in the production medium. The results in the present study agree with the study of [28] and [29].

6. Determination of molecular weight of produced lipase

Figure 3 elucidates the analysis of gel electrophoresis and molecular weight calculation of lipase in the presence of standard protein marker. The results shown the presence of a single band separated in the purified enzyme after gel electrophresis that band has been observed in all lines include the extracted crude lipase, precipitated lipase and gel filtration chromatography step with molecular weight 68KDa. In accordance the results in the present study with literature as study of [20] and [24].

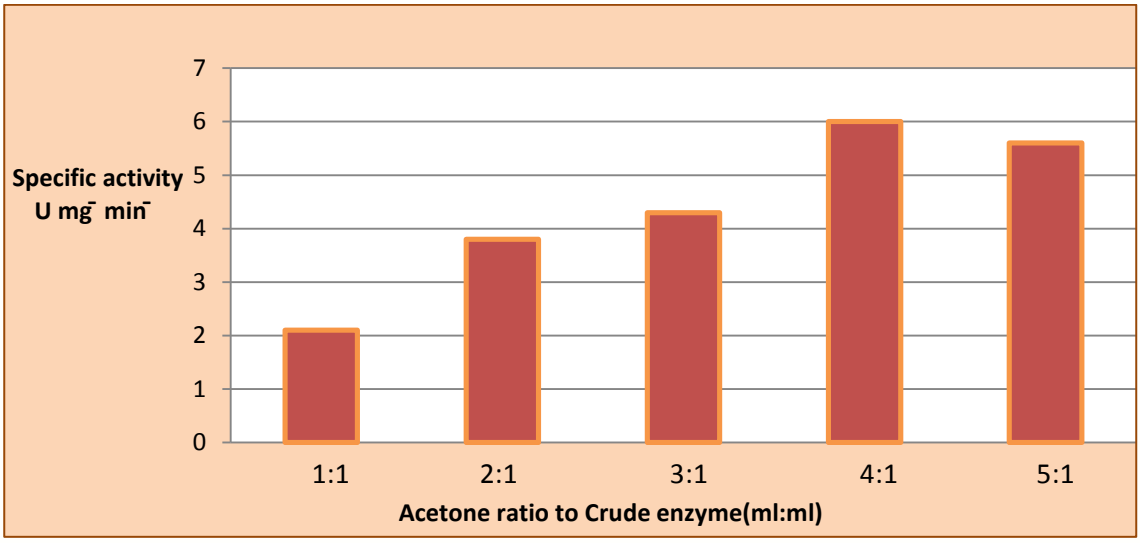

Figure (1): The precipitation of lipase by acetone ratios

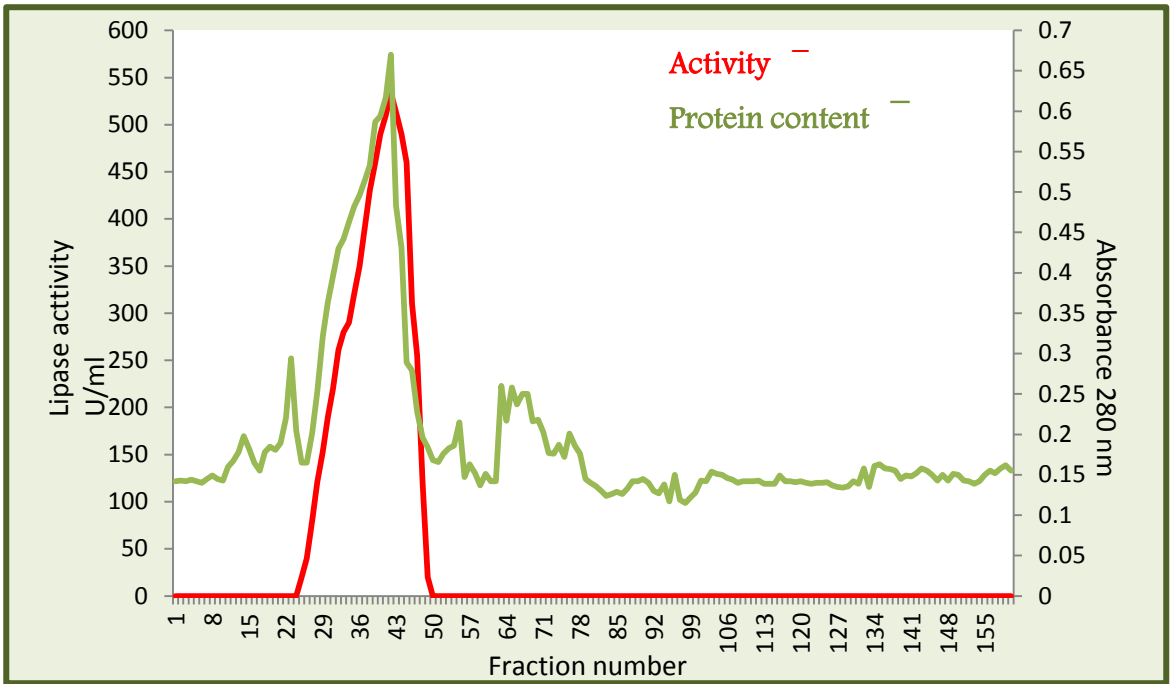

Figure (2): Gel filtration chromatography analysis of lipase using Sephadex G-150. The column (100x1.5) cm equilibrated with Tris-HCl buffer (20mM, pH 8.0) at a flow rate of 0.1ml/min.

Table (1): Purification steps of lipase from bacteria *P. aeruginosa*

Purification Steps	Volume (ml)	Activity(U/ml)		protein Content (mg/ml)	Specific activity (U/mg)	Total activity (U)	Total protein (mg)	Yield (%)	Purification fold
			Per min						
Crude extract	95	375	6.2	3.6	1.7	582.8	342	100	1
Acetone precipitation	40	864	14.4	2.4	6	576	96	98	3.5
Gel filtration chromatography	60	534	8.9	0.22	40.5	534	13.2	92	6.7

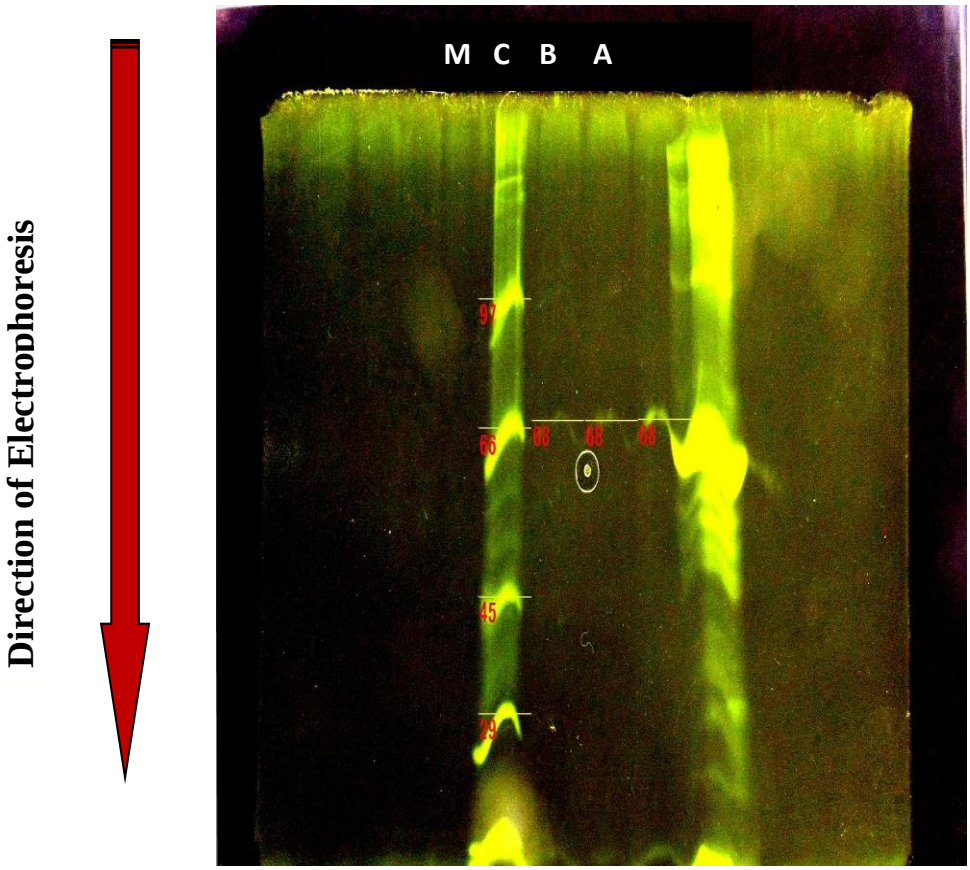

Figure (3): Analysis the polyacrylamide gel electrophoresis (SDS-PAGA) of lipase by using the CS analyzer- atto program. (A): extracted crude lipase, (B): the precipitated lipase, (C): the collected fractions were charecterized by lipase activity after gel feltration chromatograph step, (M): Marker standerd proteins.

Conclusions

The present study revealed that the oily soil samples may be used to isolate the novel strains that constitute as a part of the microbial collection for the production of lipase in the research labs and industries. The isolate *P. aeruginosa* was showed highest lipase production activity. We recommended for further studies to characterize the isolated enzyme and suggest to use the isolated enzyme in specific catalytic reaction

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