

Determination of lipase production and partial purification from local bacterial and micro algal isolates

Abstract: Lipase is enzyme capable of catalyzing and synthesis of esters formed from glycerol and long-chain fatty acids. Many types of microorganisms produce lipase, bacterial and algae lipases are important enzymes applications in various industries. Six bacterial and six algae species were collected from soil and research laboratories in Biology department/ Al-Mustansiriyah University, bacterial isolates identified as (*Enterobacter aerogenes*, *Pseudomonas alcaligenes*, *Aeromonas veronii*, *Aeromonas hydrophila*, *Serratia marcescens* and *Staphylococcus aureus*) and algae species as; (*Chroococcus minor*, *Oscillatoria tunuis*, *Anabeana sp.*, *Chlorella vulgaris*, *Nostoc sp.*, and *Chlorella ellipsoidea*). All bacterial and algae species were screened the ability for lipase production using plate assay and participation method for partial purification. Results showed a significant production of lipase for most of bacterial species, while very little to no inactivation effect for *Aeromonas hydrophila*, also there were an obvious production of *ps alcaligenes* and *S. marcescens* using plate assay method. A significant production for most of algae species for lipase enzyme while very little to no inactivation effect for *Chroococcus minor*. Protein participation by using ammonium sulphate applied to purify the enzyme in 75% saturation. Results demonstrated that *S. marcescens* bacteria and *Chlorella ellipsoidea* algae were best isolates for extracellular lipase production, and that crude enzyme has less activity than purified enzyme. Isolation and identify the Lipase enzyme production from different local bacterial and algae isolates species has a great effect on involve this enzyme in the scientific application, because of friendly for environment, non-toxic and no harmful residues.

Key words: Lipase, bacteria, algae, partial purification, production of enzyme.

Introduction

Many microorganisms and higher eukaryotes produce lipases [1]. Lipases are able to catalyze hydrolysis, esterification, trans-esterification [2, 3] and lactonization (intra-molecular esterification) [4].

Lipase producers have been isolated mainly from soil, or spoiled food material that contains vegetable oil. Lipase production from a variety of bacteria, fungi, actinomycetes, and algae, it has reported in several works [5, 6]. Some lipases expressed and secreted by pathogenic organisms during the infection. In particular, *Candida albicans* has a large number of different lipases, possibly reflecting broad lipolytic activity, which may contribute to the persistence and virulence of *C. albicans* in human tissue. In the example of lysosomal lipase, the enzyme confined within an organelle called the lysosome [7].

Fungi and bacteria may secrete lipases to facilitate nutrient absorption from the external medium (or in examples of pathogenic microbes, to promote invasion of a new host). As biological membranes are integral to living cells and are largely composed of phospholipids, lipases play important roles in cell biology [8]. The ease with which enzymes could be isolated from microbes has made both bacteria and fungi predominant sources of lipase. In eukaryotes, microalgae cells consist of cell wall, plasma membrane, cytoplasm, nucleus and organelles such as mitochondria, lysosomes, and Golgi apparatus [9]. It found to be best source for future generation fuel like biodiesel and hydrogen gas production. Microalgae highlighted as potential candidates for fuel production since they have high photosynthesis efficiency, biomass productivity, and growth rate when compared with other energy crops. In addition, the lipid content of oil-rich microalgae (e.g., *Chlorella vulgaris*) could reach 50–60% of total dry cell weight [10]. Microalgae are also an interesting source of lipases because although the presence of the enzymes known, to date no lipases have been isolated and characterized from native sources. Thus, they represent a relatively unexplored enzyme resource [11].

Materials and Methods

Isolation and identification of bacterial strains

Six bacterial species (*Enterobacter aerogenes*, *Pseudomonas alcaligenes*, *Aeromonas veronii*, *Aeromonas hydrophila*, *Serratia marcescens* and *staphylococcus aureus*) from research laboratories in Biology department / Al- Mustansiriyah University. Bacterial species were refreshing by growing on nutrient broth Oxoid (13g/ l) at 37 °C for 24 hours, then loop full of inoculated broth were streaking at nutrient agar- Oxoid (28g /l), all petri-dishes were incubated at 37 °C for 24 hours, then kept in refrigerator until day of experiments.

Serial dilution of collected bacterial strains carried out [12]. Morphological appearances of the inoculated plates (at room temperature) observed and distinct colonies were sub-cultured to obtain pure isolates, which were then maintained on NA slants and stored at 4°C for further studies. The pure bacterial isolates further identified by microscopic and biochemical examination, according to Bergey's Manual of Determinative Bacteriology [13]. All bacterial strains confirmed diagnosis using Vitek technique.

Collection of algal samples:

Samples collected from different sites (Bodies of water and soil) in Al-Mustansiriyah University. Six species identified as *Chroococcus minor*, *Oscillatoria tunuis*, *Anabeana*

sp., *Nostoc sp.* according to (14), *Chlorella, ellipsoidea* and *Chlorella, vulgaris* according to (15). Algae species scaled up using prepared media culture Chu-10 for the cultivation of green algae (Chlorophyta) [16]. As well as the media culture BG-11 for the cultivation of blue green algae (cyanobacteria) [17]. The components of media for algae listed in tables (1, 2) respectively, the preparation of Stock solutions of each salt for major nutrients (Macronutrients) and stock solutions Minor nutrient (Micronutrients) combined as follows (Table 1 and 2):

Table (1): media culture components Chu-10 used for the cultivation of green algae

Conc. g/liter	salt	Conc. gram/liter	Salt
100	a-Micronutrients	5.8	b- Macronutrients
2.86	EDTA .Na2	57.56	Sodium Meta silicate
1.81	H3BO3	10	Ca(NO3)2.4H2O
0.222	MnCl2.4H2O	25	K2HPO4
0.390	ZnSO4.7H2O	4.36	MgSO4.7H2O
0.079	Na Mo O4.5H2O	3.15	EDTA .Na2
0.049	CuSO4.5H2O	20	FeCl3.6H2O
	Co(NO3)2.6H2O		Na2Co

Table (2): media culture components BG-11 for the cultivation of blue green algae

Conc. g/liter	Salt	Conc. g/liter	Salt
2.86	a-Micronutrients	150	b-Macronutrients
1 1.8	H3BO3	30	NaNO3
0.222	MnCl2.4H2O	75	K2HPO4
0.0124	ZnSO4.7H2O	27.181	MgSO4.7H2O
0.072	NH4)Mo7O24.	6	CaCl2. H2O
0.048	4H2O(6	Citric acid
	CuSO4.5H2O	1	Ferric ammonium
	CO(NO3)2.6H2O	20	Citrate
			EDTA.Na2
			Na2CO3

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red blue-green algae (*Oscillatoria tunuis*, *Chroococcus minor* and *Anabeana sp.*) in the media BG-11 and green algae (*Chlorella vulgaris*, *Chlorella ellipsoidea* and *Nostoc sp.*) in Chu-10 media and kept in lighted incubator (25 ± 2 °C, 200µE/m²/sec and 16:8 hour Lighting: darkness) Use Batch culture for the purpose of obtaining biomass [18]. 10 ml of green algae culture and blue algae greens inculcated into 300 ml. 2.5l in autoclave prepared media added to 300 ml of algae stock solution. The final volume were incubated equipped with air through a rubber tube ends stone bubble into the pelvis [19] and placed in culture conditions as mentioned above. All algae harvested after two weeks. Algae cell were isolated using the centrifuge speeds (3000 rpm for 15 min). Sediment washing three times with distilled water, then left in the oven at 40 °C for 15 min. Collect dry output and save it in a special container in the refrigerator until use [20].

Screening of bacterial strains for lipase production on solid agar. A plate detection method containing a chromogenic substrate (Congo red) used to screen the strains for lipase producing ability. The medium used for screening has the following composition in (g/l): peptone 10; NaCl 5; Calcium chloride; 0.1; castor oil, 1ml agar, 50; Congo red, 0.5; and distilled water, 1,000 ml. The sterile medium poured plates and allows solidifying. The agar plates were spot inoculated with 100 µl of each bacterial strain and incubates for 24-27 hours. Lipolysis indicated by the appearance of clear zone of inhibition around the spot of inoculation. The diameters of the colonies and clearance zones measured after 24, 48 and 72 hours for bacteria isolates [21].

Plate Assay of Lipase Enzyme from algae:

LB Agar plates were prepared and 1% (v/v) Tween 20 added after autoclaving. Each algae strain (100 µl) tested for the production of lipase enzymes using agar plate assay. After incubated for 24, 48 hours; the presence of white clear crystal around the enzyme well confirmed the presence of lipase activity (22).

Lipase Enzyme production: The composition of production medium used in this study was: (%w/v) peptone 0.2; NH4H2PO4 0.1; NaCl 0.25; MgSO4•7H2O 0.04; CaCl2.2H2O 0.04; olive oil 2.0 (v/v); pH=7.0; 1-2 drops Tween 80 as emulsifier. Overnight cultures suspended in 5ml of sterile

distill water and used as the inoculum for pre culture. Submerged microbial cultures incubated in 500 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (150 rpm) and incubated at 36°C. After 24, 48 and 72 hours of incubation, the culture was centrifuged at 10,000 rpm for 20 min at 4°C and the cell free culture supernatant fluid was used as the sources of extracellular enzyme. Lipolytic activity was determined spectrophotometrically (24).

Measurement of Lipase Activity: Lipolytic activity was determined spectrophotometrically. The substrate solution was prepared by adding the solution A (30 mg of p-NPP in 10 ml of isopropanol) in to the solution B (0.1g of gum Arabic and 0.4 ml Triton X-100 in 90 ml of 50 mM Tris-HCl buffer, pH 7.0) with stirring until all was dissolved. The mixture of 9ml of substrate solution and 1ml of suitably diluted enzyme solution was measured for 10 min at λ=410 nm in a spectrophotometer (type). One unit of activity (U) defined as the amount of lipase required to release 1µmol of p-nitrophenol (p-NP) per min at room temperature (30 °C).

Results and discussion

Screening of lipase production using different bacterial species. Results showed a significant production for most of bacterial species with very little to no inactivation effect for *Aeromonas hydrophilia*, while there were an obvious production of *Serratia marcescens* (figure-1). Lipase production on solid agar shown in Table-3 exhibited the lipolytic activity ranged within 10mm - 40mm, at 24, 48 hours of incubation, respectively. *Enterobacter aerogenes* and *Serratia marcescens* had the highest activity at different hours of incubation. *Aromonas veronii* and *Pseudomonas alicalignese* (30mm) respectively, while *S.aureus* showed less inactivation with (10 mm) of zone of inhibition.

Similar bacterial isolates reported by Musa and Adebayo-Tayo. (2012), Deive *et al.* (2012), and Sharma *et al.* (2001). Gupta *et al.* (2004) also referenced 38 distinct bacterial sources from which common lipases derived.

Table (3): lipolytic activity of different bacterial species

Strains No.	Bacterial strain	Lipase production	IZ (mm)
1	<i>Areomonas veronii</i>	+	30
2	<i>Pseudomonas alicalignese</i>	+	30
3	<i>Aromonas hydrophilia</i>	-	0
4	<i>Enterobacter aerogenes</i>	+	40
5	<i>Serratia marcescens</i>	+	40
6	<i>S.aureus</i>	+	10

Results showed a significant lipase production for most of algae, while very little to no inactivation effect for *Chroococcus minor*, and there was an obvious production all of others (table-4).



Figure (1): lipolytic activity for different bacterial strains

Figure-2 also showed lipolytic activity of different algae strains. (Hassan *et.al.*,2006), (Anitha, and Sriman, 2012) and (Kishore, *et.al.* 2011), have reported similar bacteria isolates. (Li, *et.al.* 2011) also referenced distinct algae sources from which common.

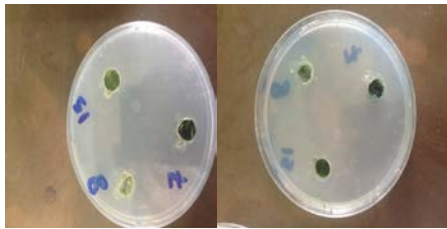


Figure (2): lipolytic activity of different algae strains

Table 4: lipolytic activity of different algae strain

Strain no.	Algae strains	Lipase production
7	<i>Chroococcus minor</i>	-
8	<i>Oscillatoria tenuis</i>	+
22	<i>Anabeana sp.</i>	+
21	<i>Nostoc sp.</i>	+
4	<i>Chlorella ellipsoidea</i>	+
15	<i>Chlorella vulgaris</i>	+

Identification of the lipase enzyme using Protein participation assay by Ammonium sulphate. Results indicated that crud enzyme has less activity than purified enzyme participated using the Ammonium sulphate (figure-3). The figure showed an increase in the optical density of the enzyme activity due to the participation of the protein, while the purified enzyme showed an increase in the optical density after two minutes. The spectrophotometric methods for lipase activity determination make use of synthetic lipase substrates transformed upon enzyme catalyzed hydrolysis into products able to be detected spectro-photometrically.

The predominant substrates are p-nitrophenyl and naphthyl esters of the long chain fatty acids, and thioesters. The lipolysis of the p-nitrophenyl esters (laurates, palmitates, oleates) gives rise to the yellow colored p-nitrophenol, measured at 405-410 nm (34).

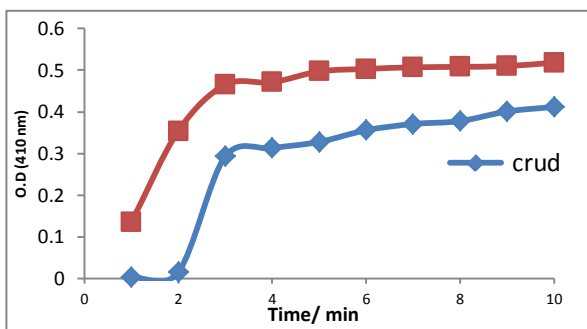


Figure (3). Enzyme activity isolated form *Serratia marcescens* bacteria analysis.

The lipase activity was estimated using spectrophotometric method for crude and purified enzyme. Figure- 4 shows the lipase activity of the different algal species. *Chlorella* algae specie showed significant difference in the lipase activity between crude and purified enzyme. a same pattern as bacterial species (22).

Conclusion

Lipase is becoming increasingly important in high-value applications in the medicinal industry and the production of variety chemicals and drugs. The current study shows that soil and bodies of water can used as good source of lipase enzyme, which may hold application in various fields. The enzyme activity and protein content varied with species and geographical area. *S. marcescens* bacteria and *Chlorella ellipsoidea* algae found to show the highest activity of the enzyme. We are currently extending our work in the characterization of lipase enzyme.

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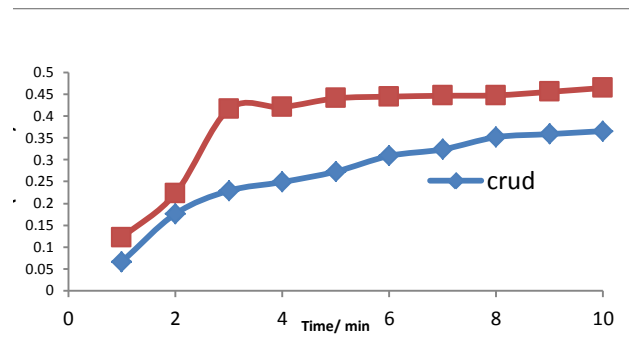


Figure (4): enzyme activity isolated from *chlorella species* analysis.

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