Research Article

# Isolation and Characterization of Lipase from Marine Algae

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**ABSTRACT**

Lipases are one of the widely used biocatalyst for various purposes and so they have wide range of applications. These water soluble enzymes have unique ability of catalyzing the hydrolysis of ester bonds in triacylglycerols. Lipase is the primary digestant used to break fats into fatty acids and glycerol. Lipase is produced by different methods and via variety of sources. Isolation of lipase is a challenging task as it should be cost effective and efficient one at industrial scale. Lipase has been isolated from marine algae which is easily available in shallow water and from culturing of the same. People are not using seaweeds as a food in India so it gives us a better chance and challenge to utilize it for different purposes. In this research work we isolated lipase from different types of seaweeds from nearby area, purified it and estimated the molecular weight of lipase. The obtained lipase was tested against different physical and chemical parameters such as effect of pH on activity and stability, effect of temperature on activity and effect of different substrates on the activity of lipase showing its optimum range and specificity for different substrates.

**Keywords:** Algae, Enzyme, Lipase.

## INTRODUCTION

Lipass are acyl hydrolases and water soluble enzymes that play a key role in fat digestion by cleaving long chain triglycerides into polar lipids. Because of an opposite polarity between the enzyme (hydrophilic) and their substrates (lipophilic), lipase reaction occurs at the interface between the aqueous and the oil phases.[1]

Lipases (EC.3.1.1.3, triglycerol acylhydrolases) are a group of enzymes, which have the ability to hydrolyze triglycerols at an oil-water interface to release free fatty acids and glycerols.[2] Lipases are present in plants, animals and microorganisms.[3] Lipases catalyze a wide range of reactions, including hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification and aminolysis.[4]

Their biotechnological potential is relying on their ability to catalyze not only the hydrolysis of a given triglyceride, but also its synthesis from glycerol and fatty acids. Therefore, microbial lipases have many industrial applications.[5] The temperature stability of lipases are the most important characteristic for industrial use.[6]

Isolation of lipase from seaweeds open novel routes for industrial production. Seaweeds or benthic marine algae are the group of plants that live either in marine or brackish water environment. Like the land plants, seaweeds contain photosynthetic pigments and with the help of sunlight and nutrient present in the seawater, they photosynthesize and produce food.[7]

Electrophoresis is a bioanalytical tool used in fundamental research for the isolation and identification of high molecular weight biomolecules. Most biopolymers, such as proteins and nucleic acids, are charged, so they can be separated and by electrophoretic methods.[8]

## MATERIALS AND METHODS

### Collection and processing of samples

The samples were collected from three different places viz. Covelong beach (Chennai), Rock beach (Pondicherry), Mammalapuram beach (Mahabalipuram). The samples were dried and ground. The powdered sample was used for further process.

### Quantification of Protein

The protein content was estimated using Bradford method at 590nm.[9]

### Precipitation of Protein content by Ammonium sulphate

Ammonium sulphate to be added was weighed (6.63g for 100ml). Ammonium sulphate was added slowly while stirring. The samples were centrifuged at 10,000g for 15min at 4°C. The pellet containing the precipitated protein was dissolved in a TCA buffer for further analysis and purification.[10]

### Partial Purification of Lipase by Sephadex G-100

The columns were packed with Sephadex grade 100. The crude extract was suspended in TCA buffer and passed through column. The eluent was collected and assayed for enzyme activity.[11]

### Enzyme assay using castor oil as substrate

Lipase activity was determined by titrometry method using castor oil emulsion. It was prepared by mixing 25 mL of castor oil and 75 mL of 7% Arabic gum solution in a homogenizer for 2 min. The reaction mixture containing 5 mL of castor oil emulsion, 4 mL of 50 mM Tris-HCl buffer, pH 7.5, 1 mL of 110 mM $CaCl_2$ and 1 mL of partially purified enzyme solution was incubated at 37°C for 30

min under orbital shaking at 200 rpm. The reaction was immediately stopped after the incubation period by the addition of 15 mL chloroform:ethanol mixture (2:1 v/v), and the released free fatty acids were titrated with 50 mM NaOH. The amount of NaOH was noted.[12]

### Effect of pH on activity and stability

The pH effect on activity and stability was studied by castor oil assay in a pH range of 3.5-11.0 using different citrate and phosphate buffers at 50 mM concentration. For stability, 1 mL of partially purified enzyme was mixed with 1 mL of respective buffer and incubated overnight at 4ºC in refrigerator. After that, the assay was performed as described for castor oil assay.[12]

### Effect of temperature on activity and stability

Effect of temperature on lipase activity was studied by carrying out the enzyme reaction at different temperatures in the range of 10-70°C at pH 7.5 using Tris-HCl buffer (50 mM). The thermo stability of lipase was tested by pre-incubating the enzyme solution at different temperatures ranging from 20 to 90°C for 1 h. After that, the assay was performed.[12]

### Substrate specificity

About 2ml of different oils and fatty substances (castor oil, sunflower oil, soybean oil, gingelly oil and milk cream) were taken and neutralized to pH 7.0. The solution was stirred well with 25ml of sterile distilled water in the presence of 100mg bile salts till an emulsion is formed. About 2g gum acacia was added to hasten emulsification. 20ml of substrate was taken in a 500ml beaker and 5ml of phosphate buffer was added and the beaker was placed on the top of magnetic stirrer cum hot plate maintained at 35°C. The pH was adjusted to 7.0 and enzyme extract 0.5ml was added, immediately the pH was noted and the timer was set at t=0. After every 2min the pH of the sample was recorded with subsequent addition of NaOH. Total amount of NaOH required for bringing the pH at its initial value was noted.[13]

### Lipase Activity meq/min/g sample

= (vol. of alkali consumed * strength of alkali)/ (Wt. Of sample in g * time)

### Molecular weight determination

In order to find out the approximate molecular weight of lipase enzyme, SDS-PAGE was run with the protein marker. The partially purified enzyme extract from 6 different species were used as a sample. Separation was carried out in Electrophoresis device. 12% polyacrylamide gel was prepared according to the protocol.[14, 15]

## RESULTS AND DISCUSSION

The enzyme lipase was isolated and characterized from six species of marine algae collected from the coastal areas of Tamil Nadu and Puducherry.

**Table 1:** Protein estimation of crude and purified samples

| Species Name | Protein content (µg/ml) | |
|---|---|---|
| | crude extract | purified sample |
| *Ulva lactuca* (Puducherry) | 92 | 82 |
| *Ulva fasciata* (Puducherry) | 97 | 82 |
| *Enteromorpha compressa* (Puducherry) | 87 | 77 |
| *Chaetomorpha antenna* (Puducherry) | 107 | 92 |
| *Ulva lactuca* (Mahabalipuram) | 92 | 77 |
| *Gelidium pusillum* (Kovalam) | 112 | 92 |

The result in Table 1 shows the estimation of protein content with the help of Bradford reagent. The analysis of 6 different species from different geographical area of Southern east coast showed difference in protein content. The highest protein content was observed in *Gelidium pusillum* (Kovalam) and *Chaetomorpha antenna* (Puducherry) while lowest was found in *Ulva lactuca* (Mahabalipuram) and *Enteromorpha compressa* (Puducherry). The change in geographical area leads to change in protein concentration of the respective species depending upon surrounding environment and other physical factors.

Activity is the ability of an enzyme to catalyze a particular reaction under certain conditions. Higher the activity, stronger is the catalytic ability of an enzyme. The results in Table 2 showed that the highest activity was conferred to *Gelidium pusillum* from Covelong beach while lowest activity was observed in *Ulva lactuca* from Mahabalipuram. Lipase extracted from *Gelidium* hydrolyzed castor oil with less dosage when compared to others.

Physical factors such as pH and temperature affect the activity and stability of an enzyme. Thus change in the activity leads to catalytic ability of an enzyme. The optimum pH range observed was between 7-8 at normal temperature (Table 3, Figure 1). The maximum activity was observed at pH 7.5.

The optimum temperature range was 30-40ºC at constant pH 7.5 (Table 4, Figure 2). Most of the species showed maximum activity at 40ºC while *Ulva lactuca* showed at 30ºC. All the test samples were analyzed against standard lipase.

The change in substrate level and type of substrate showed different level of hydrolysis. From the results in Table 5 and Figure 3, the average highest values were observed for *Gelidium* and substrate as castor oil.

The electrophoresis results showed that the lipase enzyme obtained from marine algae was having molecular weight of 45-60 kDa. The comparison was

made with the standard lipase as a molecular marker. The partially purified enzyme samples didn't show the clear band with two species but the range of the lipase can be interpreted from the gel documentation.

**Table 2:** Enzyme activity at Normal Temperature

| Name of sample | Initial pH | After addition of enzyme | Vol of NaOH (mL) | Time (min) | Final pH | Wt of pellet (g) | Activity meq/min/g |
|---|---|---|---|---|---|---|---|
| *Ulva lactuca* (Puducherry) | 7.09 | 5.4 | 7.9 | 10 | 7.03 | 0.69 | 0.057 |
| *Ulva fasciata* (Puducherry) | 7.07 | 5.57 | 7.6 | 9 | 7.00 | 0.72 | 0.058 |
| *Enteromorpha compressa* (Puducherry) | 7.01 | 5.23 | 8.3 | 11 | 7.04 | 0.6 | 0.062 |
| *Chaetomorpha antenna* (Puducherry) | 7.00 | 5.30 | 8.0 | 9 | 7.01 | 0.65 | 0.068 |
| *Ulva lactuca* (Mahabalipuram) | 7.01 | 5.43 | 7.7 | 10 | 7.06 | 0.76 | 0.050 |
| *Gelidium pusillum* (Kovalam) | 7.05 | 5.1 | 8.6 | 8 | 7.06 | 0.62 | 0.086 |

**Table 3:** Effect of pH on lipase activity and stability at normal temperature

| Name of sample | pH range | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4.0 | 5.5 | 6.5 | 7.5 | 8.5 | 9.0 | 11.0 |
| Standard Lipase | 0.042 | 0.056 | 0.063 | 0.084 | 0.085 | 0.072 | 0.055 |
| *Ulva lactuca* (Puducherry) | 0.033 | 0.062 | 0.069 | 0.066 | 0.052 | 0.043 | 0.029 |
| *Ulva fasciata* (Puducherry) | 0.022 | 0.052 | 0.062 | 0.071 | 0.066 | 0.061 | 0.054 |
| *Enteromorpha compressa* (Puducherry) | 0.029 | 0.047 | 0.059 | 0.063 | 0.061 | 0.054 | 0.047 |
| *Chaetomorpha antenna* (Puducherry) | 0.020 | 0.056 | 0.073 | 0.073 | 0.069 | 0.059 | 0.033 |
| *Ulva lactuca* (Mahabalipuram) | 0.037 | 0.048 | 0.053 | 0.056 | 0.051 | 0.047 | 0.034 |
| *Gelidium pusillum* (Kovalam) | 0.026 | 0.057 | 0.079 | 0.081 | 0.074 | 0.068 | 0.042 |

**Table 4:** Effect of temperature on lipase activity

| Name of sample | Temperature range (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 30 | 40 | 50 | 60 | 70 |
| Standard Lipase | 0.043 | 0.059 | 0.061 | 0.053 | 0.039 | 0.010 |
| *Ulva lactuca* (Puducherry) | 0.039 | 0.062 | 0.065 | 0.059 | 0.049 | 0.033 |
| *Ulva fasciata* (Puducherry) | 0.010 | 0.044 | 0.052 | 0.036 | 0.033 | 0.029 |
| *Enteromorpha compressa* (Puducherry) | 0.023 | 0.062 | 0.066 | 0.060 | 0.044 | 0.028 |
| *Chaetomorpha antenna* (Puducherry) | 0.037 | 0.069 | 0.073 | 0.061 | 0.041 | 0.031 |
| *Ulva lactuca* (Mahabalipuram) | 0.021 | 0.056 | 0.052 | 0.048 | 0.039 | 0.020 |
| *Gelidium pusillum* (Kovalam) | 0.041 | 0.071 | 0.076 | 0.061 | 0.046 | 0.032 |

**Table 5:** Effect of substrate on lipase activity

| Name of sample | Substrate | | | | |
|---|---|---|---|---|---|
| | Castor oil | Soybean oil | Sunflower oil | Gingelly oil | Milk cream |
| Standard Lipase | 0.081 | 0.073 | 0.093 | 0.069 | 0.058 |
| *Ulva lactuca* (Pondicherry) | 0.072 | 0.061 | 0.069 | 0.057 | 0.054 |
| *Ulva fasciata* (Pondicherry) | 0.063 | 0.066 | 0.069 | 0.059 | 0.043 |
| *Enteromorpha compressa* (Pondicherry) | 0.059 | 0.055 | 0.062 | 0.051 | 0.041 |
| *Chaetomorpha antenna* (Pondicherry) | 0.079 | 0.071 | 0.077 | 0.063 | 0.056 |
| *Ulva lactuca* (Mahabalipuram) | 0.063 | 0.058 | 0.060 | 0.055 | 0.047 |
| *Gelidium pusillum* (Kovalam) | 0.080 | 0.071 | 0.079 | 0.069 | 0.061 |

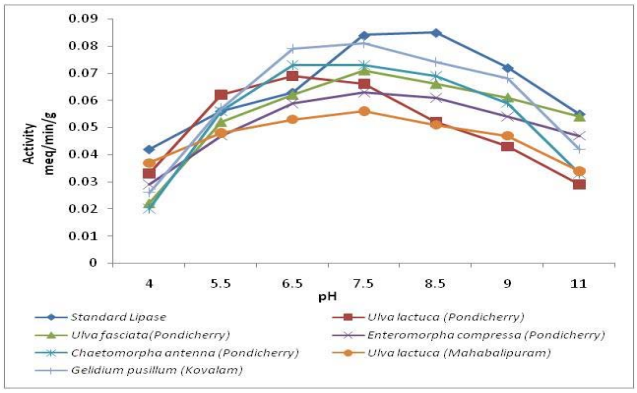


**Figure 1:** Optimum pH range observed for highest lipase activity

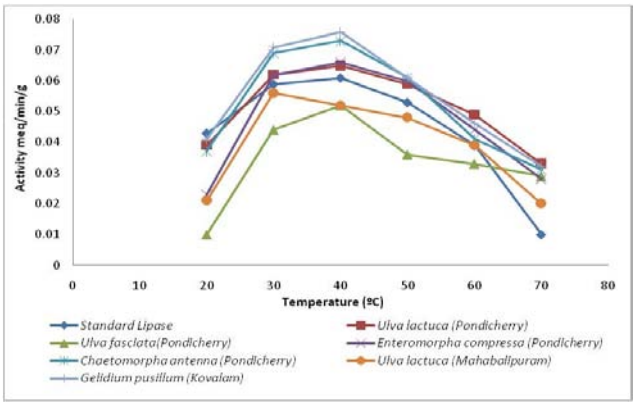


**Figure 2:** Optimum temperature range observed for highest lipase activity

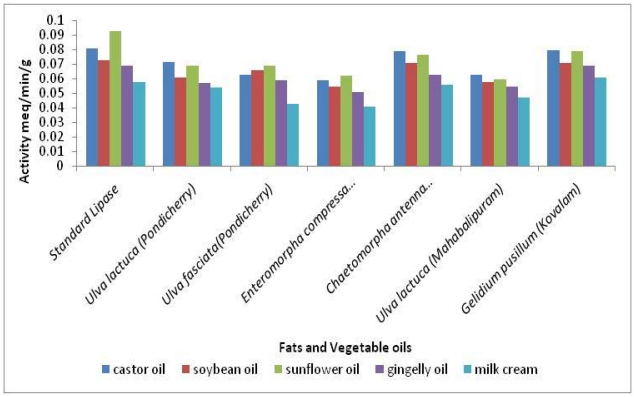


**Figure 3:** Level of hydrolysis activity from different fatty substances and vegetable oils

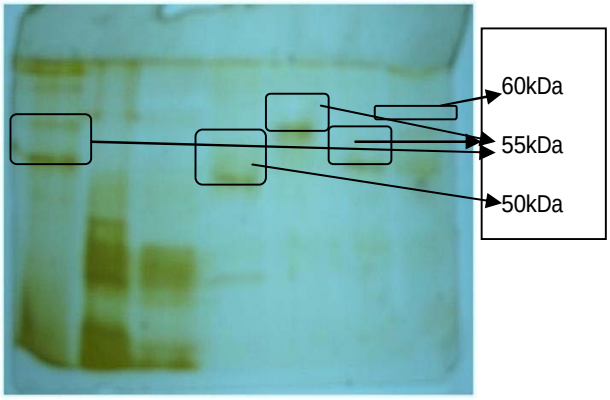


**Figure 4:** SDS-PAGE of the 6 different species

Lane 1 - *Ulva lactuca* (Puducherry), Lane 2 – *Enteromorpha compressa* (Puducherry), Lane 3 – *Gelidium pusillum* (Covelong), Lane 4 – Std lipase, Lane 5 – *Ulva fasciata* (Puducherry), Lane 6 – *Ulva lactuca* (Mahabalipuram), Lane 7 – *Chaetomorpha antenna* (Puducherry)

## CONCLUSION

The major purpose of the study carried out was to investigate, characterize and purify the lipase enzyme from various marine algae. The enzyme extracted from different marine algae showed positive results for lipase assay protocols confirming the presence of lipase.

According to the results the highest enzyme activity was shown by *Gelidium pusillum.* The activity was analyzed against different parameters like temperature, pH and substrate specificity. The optimum pH was found out to be 7.5 while the temperature was 40ºC. Although the pH and temperature were optimum at certain point but lipase show stability over wide range of pH and temperature. Highest enzyme activity was found against castor oil among other fatty substances including vegetable oils and milk cream.

SDS-PAGE used for the estimation of molecular weight showed more or less same molecular weight as the standard sample. So the lipase obtained can also be used for laboratory and industrial use depending upon the purity of enzyme. Further purification may lead to the exact molecular weight of the lipase obtained, thus the selectivity will be increased and its further analysis may show better results.

Lipase has been previously isolated from different microbes including bacteria and fungi. Here we attempted isolation of lipase from marine algae as it is readily available in bulk quantities. The protocol used for isolation and purification were suitable for laboratory purpose but for future perspective it should be optimized according to the requirement of industry. The use of lipase in industry from algae is still unknown. Further research is needed in this field.

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