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Optimization of Lipase Enzyme Activity Produced By Bacillus Amyloliquefaciens Isolated From Rock Lobster Panlirus Homarus

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Abstract: *The production of extracellular lipase (triacylglycerol hydrolase) is a widely distributed phenomenon among eukaryotic and prokaryotic microorganisms. Microbial lipases have mostly been employed in food and detergent industry for purposes such as the ripening of cheese and an additive to laundry detergent. Lipase producing organisms were isolated from Rock Lobster (**Panlirus homarus**). **Bacillus amyloliquefaciens** was found to be the highly active strain for lipase enzyme production. The most suitable for lipase production is fish bone and the maximum amount of paranitrophenyl palmitate released is found to be 0.84 µg/ml/mints at 48hrs of incubation. The optimum temperature and pH for the organism was found to be 47°C and 9, the amount of paranitrophenyl palmitate released was recorded as 0.89 µg/ml/min and 0.74 µg/ml/min for various substrates such as Glycerol, Olive Oil, Coconut Oil and Fishbone. Lipase production was obtained at different substrate concentration and the bacterial species yield maximum production of lipase and it was analysed by spectrophotometric method.*

Keywords: *Rock lobster (Panlirus homarus**)** – Bacillus amyloliquefaciens , Optimization of Lipase enzyme with different substrates..*

I. INTRODUCTION

Lipases are enzymes capable of hydrolyzing the ester bonds of water-insoluble substrates at the interface between substrate and water. It is well known that the reaction is reversible and this enzyme can catalyse ester synthesis and transesterification since lipases can catalyse numerous different reactions they have been widely used in industrial applications, such as in food, chemical, pharmaceutical and detergent industries [1]. Many microorganisms are known as good producers of extracellular lipases [2].

Lipases occur widely in bacteria, yeast and fungi [3]. Most of the lipase research focuses on the production of extracellular lipases through a wide variety of microorganisms. Studies on the production of extracellular lipases with *Bacillus* have shown variations among different strains. However, the requirement for lipid carbon source remains essential for enzyme production. Lipase producing organisms are isolated from Lobster (***Panlirus homarus)*** and that the wild and cultured lobsters harbours a diverse bacterial flora which includes the dominant generas like *Aeromonas, Pseudomonas, Bacillus, E.coli, Salmonella and Vibrio* [4]. These organisms are having the ability to produce enzymes. These enzymes are produced during the utilization of certain nutrients such as proteins, lipid and carbohydrate. Lipases have potential applications in detergent, oleo chemical, paper manufacturing, cosmetics, pharmaceuticals, and agrochemical industries. They are also employed in organic chemical processing, biosurfactant synthesis, nutrition and biomedical sciences [5].

Most of the commercial lipases produced are utilized for flavour development in dairy products and processing of other foods, such as meat, vegetables, fruit, baked foods, milk products and beer. Lipases are extensively used in dairy industry for the hydrolysis of milk fat. The dairy industry uses lipases to modify the fatty acid and chain lengths, to enhance the flavours of various cheeses. Lipases enzyme also accelerates cheese ripening and the lipolysis of butter, fat and cream [6]. The objective of this study was the production of lipase and characterization of the enzyme with regards to its stability in relation to temperature, P^H and the optimization of the temperature and P^H conditions for obtaining higher lipase activity.

II. MATERIAL AND METHODS

2.1 Collection of Sample:

Lobster sample was collected in a sterile bag from the fish market near Kanyakumari, Tamil Nadu, India and brought to the laboratory within 15 minutes.

2.2 Isolation of Intestinal Flora of Lobster:

The intestine of the lobster was dissected out aseptically and homogenized with 0.089% of NaCl solution. The homogenate was serially diluted and spread on sterilized nutrient agar plates and incubated at 37^0C for 24 hours. The colonies were counted for TVC (Total viable Count). The dominant colonies were isolated and streaked in nutrient agar slants as master culture.

2.3 The Composition of Production Medium Used For Lipase Production:

The production medium was prepared for lipase production by the ***Bacillus amyloliquefaciens*** in the following composition.

- Peptone - 3% (w/v)
- Yeast Extract - 1% (w/v)
- Sodium Chloride - 0.5% (w/v)
- Olive Oil - 1% (w/v)
- P^H - 7

2.4. Assay of Lipase Enzymes

2.4.1. Optimization of Fermentation Media:

The fermentation media prepared was optimized with following factors such as Incubation period, P^H , Temperature and Lipid Substrates.

2.4.1.1. Optimization by Incubation Period:

The organisms were subjected to different incubation periods such as 24, 48 and 72 hours using production media.

2.4.1.2. Optimization by different P^H

The microorganisms isolated from the rock lobster were incubated in the production media containing different P^H varied from 5, 7 & 9.

2.4.1.3. Optimization by different temperature

The mircroorganism in the production media was incubated at different temperature 27^0C, 37^0C, and 47^0C.

2.4.1.4. Optimization by Lipid Substrate

In order to optimize the enzyme lipase different lipid substrates were used, such as, Glycerol, Olive oil, Coconut oil, Fish Bone and chicken intestine.

2.4.1.5. Enzyme Assay

Microbial Culture was assayed for lipase enzyme activity using Spectrophotometric method and results were recorded.

2.5. Principle

In the present investigation one unit (U) of lipase activity was defined as the amount of enzyme solution liberating 1µmol of p-nitrophenol per minute under standard assay conditions.

2.6. Reagents Required

To carry out the study following reagents were required such as, Reaction Buffer (500µl) (It contains the mixture of 50 mM Tris Hcl (P^H 9.0) and Triton X-100), 2-Propanol (20µl) p-nitrophenylpalmitate (P^{NPP})

The spectrophotometric method, using P-nitrophenyl Palmitate as substrate was applied for rapid and routine measurement of the lipase activity. Enzyme or blank solution (480µl) was added to the reaction buffer (500µl) which has 50mM Tris-HCI (P^H 9.0) with variable concentrations of Triton x-100. The content was incubated at 25^0C for 5 minute and ten millimolar P^{NPP} in 2-propanol (20µl) was added to the enzyme buffer solution and shaken well. The mixture was emulsified for 2 minutes at 50^0C. The progress of the reaction was followed by monitoring the change in the absorbance at 400nm over a period of 5 minutes at 50^0C using Perkin-Ealmer Spectrophotometer. The molar extinction coefficient of P-nitrophenol ($E=16.900M^{-1}$ Cm^{-1}) was estimated from the absorbance measured at 400 nm of standard solutions of P^{NP}.

III. RESULTS

The enzymatic activity was assayed with different substrates like Glycerol, Olive oil, Coconut oil and Fish bone. The production of lipase was observed at varying pH (5,7 & 9), temperature (27^0C, 37^0C & 47^0C), substrate concentration (0.1, 0.2, 0.3, and 0.4) and at different time intervals (24, 48, and 72 hrs). Lipase producing organisms was isolated from rock lobster and was noted as k_1.It was identified as lipase positive organism by comparing with Bergey's Manual of Determinative Bacteriology and this organism was the highly active strain for lipase production.

The effect of lipase production by the *Bacillus amyloliquefaciens (k1) w*ith various substrates (glycerol, olive oil, coconut oil and fish bone) at different incubation periods (24hrs, 48hrs and 72hrs) were carried out and the results were indicated in Fig.1. The most suitable substrate for lipase production was identified as fish bone, and the maximum amount of paranitrophenyl palmitate released was recorded as 0.84 µg/ml/min at 48 hrs of incubation period. The minimum amount of paranitrophenyl palmitate was released with olive oil was recorded as 0.10 µg/ml/min at 48hrs of incubation time.

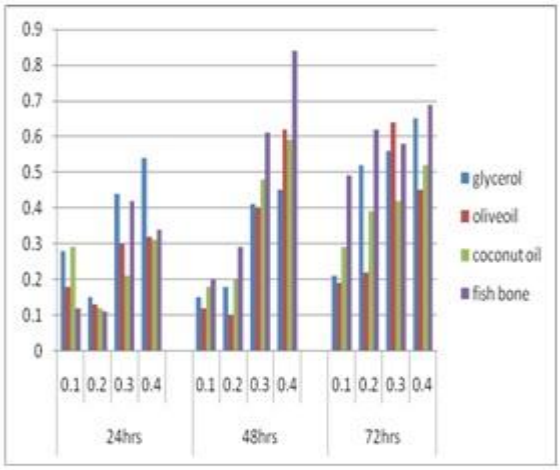

Fig.1 Effect of different substrates on lipase production by *Bacillus amyloliquefaciens (K1)*

The effect of pH on lipase production at various incubation time intervals have been carried out and the results were indicated in Fig. 2. At 48hrs of incubation at pH 9, the *Bacillus amyloliquefaciens (k1)* showed the maximum production lipase and the amount of paranitrophenyl palmitate released was recorded as 0.89 µg/ml/min, whereas the minimum production was noted at pH 5 at 24hrs of incubation period and the amount of paranitrophenyl palmitate released was recorded as 0.31 µg/ml/min.

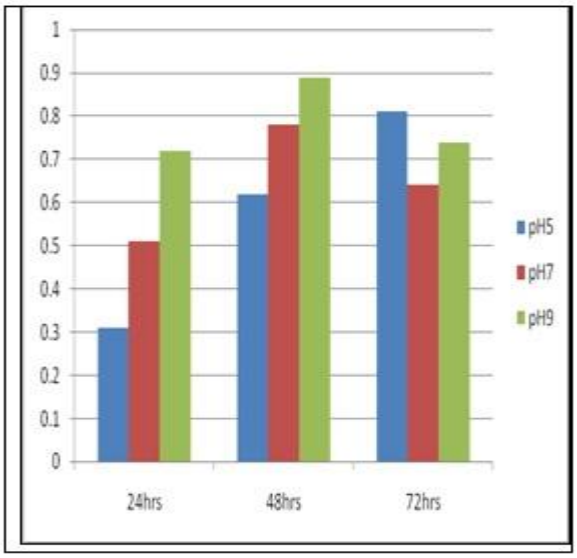

Fig.2 Effect of different pH on lipas production by *Bacillus amyloliquefaciens (K1)*

The effect of different temperatures on lipase production at various time intervals was shown in Fig.3. At 72hrs of incubation at 47^{0}C, the *Bacillus amyloliquefaciens (k1)* showed a maximum production and the amount of paranitrophenyl palmitate released recorded wsa 0.74 µg/ml/min, whereas the minimum production observed at 37^{0}C at 24hrs and the amount of paranitrophenyl palmitate released was recorded as 0.43 µg/ml/min.

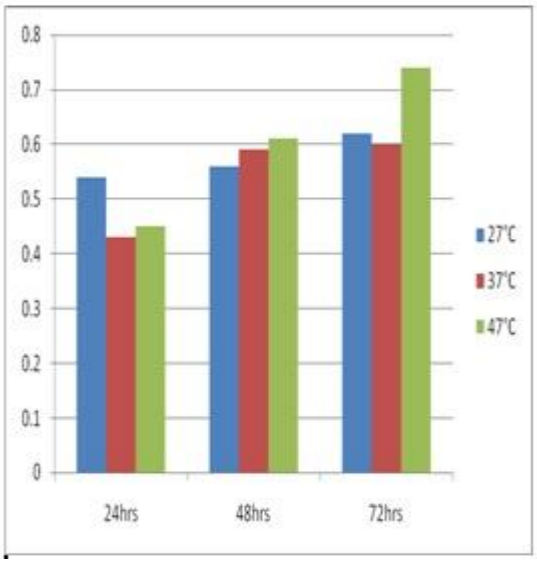

Fig.3 Effect of different temperature on lipase produnction by *Bacillus amyloliquefaciens (K1)*

IV. DISCUSSION

Lobsters harbors a diverse bacterial flora and most of the microorganisms produce enzymes.Lipase producing microorganism isolated from the lobster was identified as *Bacillus amyloliquefaciens* (k1). The lipase enzyme activity was assayed with varying pH , temperature and susbstrates at different time intervals In the present study the effect of lipase enzyme activity with the fish bone as a substrate showed the maximum production (0.84 µg/ml/min) at 48hrs of incubation and the minimum amount of paranitrophenyl palmitate released in olive oil as a substrate was recorded as 0.10 µg/ml/min at 48hrs of incubation. Among the various substrates, gingili oil cake and wheat bran were found to be the best substrates [7]. Whereas in the present investigation, the fish bone used as substrate was found to be the best with a high enzyme production.

At 48hrs of incubation with pH 9, the *Bacillus amyloliquefaciens (k1)* showed the maximum production and the amount of paranitrophenyl palmitate released was recorded was 0.89 µg/ml/min and the minimum production at pH 5 at 24hrs the amount of paranitrophenyl palmitate released was 0.31 µg/ml/min.The lipase enzyme was active in the range of pH 7.5-9.5, and the maximal activity was observed at pH 9.0. It is a characteristics that the most microbial lipases, the optimum pH falls on the alkaline side. In present study the similar trend was observed that pH was optimum and it falls on the alkaline side [8].

At 72hrs of incubation at 47^{0}C the *Bacillus amyloliquefaciens (k1)* showed the maximum production and the amount of paranitrophenyl palmitate released was observed as 0.74 µg/ml/min and the minimum production noted at 37^{0}C at 24hrs, and the amount of paranitrophenyl palmitate released was recorded as 0.43 µg/ml/min.In contrast, in the present study, the P^{NPP}-hydrolysing activity had a clear temperature which is "optimum" at 37^{o}C even though the enzyme was fully stable up to 80^{o}C for at least 15 min, which is much longer than the duration of the activity assay [9].

The overall properties showed by *Bacillus amyloliquefaciens (k1)* indicated that the cloned lipase is a mesophilic enzyme acting on a broad range of pH that becomes inactivated only by a limited number of lipase inhibitors. In the present study, pH was varied using different substrates concentration [10]. In connection with their biochemical properties, both lipolytic enzymes display different substrate specificities [11]. In the present study the substrate specificities are done using Glycerol, Coconut Oil, Olive Oil, and Fish Bone as substrates.

The cell produces lipases in order to obtain the energy required to form new cells from the available carbon sources and at the same time it does a normal metabolic activity irrespective of growth [12]. Comparing results of lipolytic activities with those of other research studies is difficult owing to the different methodologies used by different groups. However, comparing the results obtained in this work with those reported using the same methodology and reaction substrate.

Glucose, Olive Oil, Peptone and $Fecl_3$ $6H_2O$ were found to have more significance on lipase production by *Candida rugosa*.[13]. In the present study glucose, olive oil, coconut oil and fish bone are used as substrates and among that fish bone showed the highest enzymatic production.

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