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Effect of Various Physio-chemical Parameters on Production of Lipase by *Staphylococcus pasteuri*

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In the present study *Staphylococcus pasteuri* producing lipase enzyme was isolated from spoilt apple and identified by 16S rRNA sequencing method. Optimization of conditions for the maximum lipase production was carried out. Lipase production was studied in YPD broth containing 1% olive oil, mustard oil, palm oil, coconut oil and groundnut oil under shaker conditions. Incubation time ranging from 24 to 144hrs at a temperature range of 10°C – 55°C was used. pH of the medium varying from 4 – 10 containing various metal ions like Cd^{2+}, Hg^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+} and Mg^{2+} was used. Lipase production was measured in terms of enzyme activity. Maximum lipase production was achieved at a temperature of 37°C, pH 8.0 after 120 hrs of incubation in presence of $CaCl_2$ using 1% olive oil as the substrate.

Key words: Lipase, *Staphylococcus pasteuri,* shaker conditions, olive oil.

Microbial lipases are a group of biotechnologically valuable enzymes. Due to their various applied properties, they are described to be highly versatile in nature. They have slowly emerged as one of the leading biocatalysts having definite potential in exploiting the less utilized lipid technology.[1] Lipases are ubiquitous in nature and can be obtained from a variety of animals, plants, or microorganisms with very different molecular weights, optimum pH, temperatures, and substrate and reaction specificities. The industries specially focus on microbial lipases due to their selectivity, stability, and broad substrate specificity.[2, 3] Solid substrate and submerged fermentation are the methods employed for large scale production of lipases. Fungal species are cultured in solid-state fermentation, while for yeasts and bacteria submerged fermentation is preferred.[2]

Submerged fermentation is the most common fermentation technique used for the production of industrial enzymes. The yield of lipase during submerged fermentation depends on many factors like the microorganism used, nutritional components, physical conditions such as pH, temperature, aeration, agitation rate etc.

Optimum pH and temperature supporting maximum enzyme activity plays a crucial role in deciding the application of lipases. For example if the lipase enzyme withstands alkaline conditions it is suitable for detergent formulations while acidic lipase is preferable for dairy industry.[4, 5]

Microbial lipases are highly diversified in their enzymatic properties and substrate specificity, which make them very attractive for

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industrial applications. It is expected that in the next few years' lipases will benefit from their versatility and continued penetration into the detergent and cosmetics market.[6] Increasing demands of lipases has led to the commercialization of lipase production and the search for new enzyme sources, due to which it is a prime area of interest for microbiologist, process engineers and biochemists.

MATERIALS AND METHODS

Culture was isolated from spoilt apple using Tributyrin agar and identified using 16s rRNA sequencing method as *Staphylococcus pasteuri*. All chemicals and reagents used were obtained from LOBA Chem and SRL diagnostics, India.

Enzyme preparation

Yeast extract peptone dextrose broth (YPDB) containing 1% olive oil was used for the production of the lipase enzyme. 100 ml of YPDB was inoculated with 1ml of overnight culture of *Staphylococcus pasteuri* and incubated under shaker conditions. Samples withdrawn were centrifuged and the supernatant was used as the enzyme preparation in the assay**.**

Assay of lipase enzyme

Lipolytic activity of the bacterial isolate was measured, by titrimetric method[7] using olive oil as substrate. The reaction mixture was prepared containing 4ml of olive oil emulsion composed of 2gms of olive oil + 2gms of gum acacia in 200ml of sodium-phosphate buffer (pH 8.0). To this mixture 5ml of citric acid buffer (pH 6.2) + 1ml of enzyme solution was added and the flasks were incubated at 37°c for 15 minutes. After incubation enzyme activity was stopped by adding 10ml of methanol & liberated free fatty acids were titrated against 0.05N NaOH using phenolphthalein as indicator. For blank 10ml of methanol is added prior to adding enzyme solution.

$$\text{Units/ml / hour} = \frac{(\text{NaOH})(\text{Molarity of NaOH})(1000)(4)(\text{df})}{\text{Volume of enzyme used (ml)}}$$

(NaOH) = Volume (in milliliters) of NaOH used for titration of the test solution minus volume (in milliliters) of NaOH used for titration of the Blank.

Molarity of NaOH = 0.05 M.

1000 = Conversion factor from milliequivalent to microequivalent

4 = Time conversion factor

df = Dilution factor

One unit of the enzyme will hydrolyze 1.0 microequivalent of fatty acid from a triglyceride in one hour at pH 8.0 at 37°C.

Optimization of conditions for maximum lipase production

The effect of different substrates on lipase production was studied using YPDB containing 1% oil (olive oil, coconut oil, palm oil, mustard oil, groundnut oil) at various time intervals of 24, 48, 72, 96, 120 & 144 hrs.[8] Lipase production was reported in terms of enzyme activity. Samples were withdrawn and assayed for enzyme activity. Substrate exhibiting maximum lipase activity was selected for other parameter studies. The optimum temperature and pH for lipase production was determined using YPDB containing 1% olive oil incubated at the temperature range from 10^0C - 55^0C with pH range from 4 – 10. Samples were withdrawn and assayed for enzyme activity after 120 hrs. Effect of different metal ions on lipase production was studied using YPDB supplemented with 0.05 gm of $CdCl_2$, $HgCl_2$, $ZnCl_2$, $CaCl_2$, $MgCl_2$, and $FeCl_2$ respectively. After 120 hrs of incubation the sample was analyzed for enzyme activity.[9, 10]

RESULTS AND DISCUSSION

In the present study, lipase producing *Staphylococcus pasteuri* was isolated and used for optimizing the conditions for maximum lipase production.Better growth and higher enzyme activity was obtained on medium supplemented with olive oil, mustard oil and groundnut oil giving an activity of 220 U/ml, 200U/ml and 200 U/ml

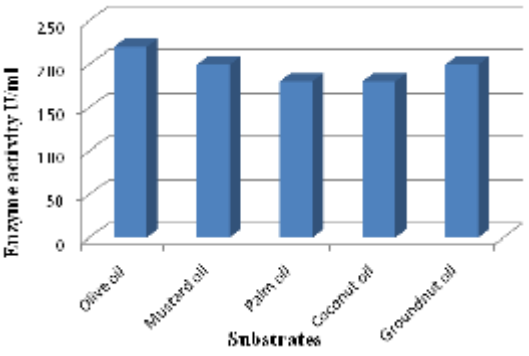

Fig. 1. Effect of substrates on lipase production

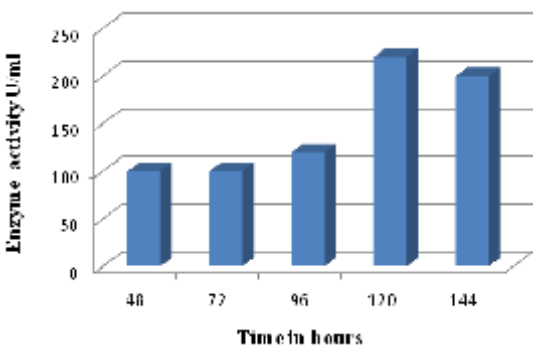

Fig. 2. Effect of incubation time on lipase production

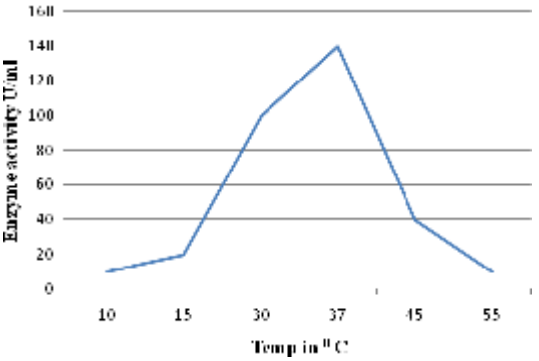

Fig. 3. Effect of temperature on lipase production

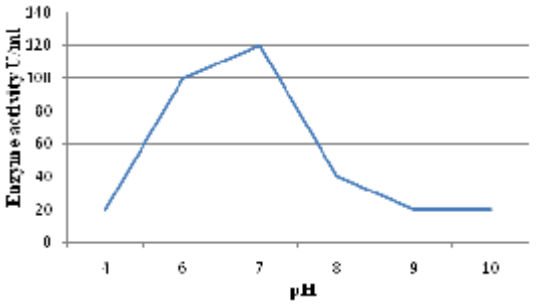

Fig. 4. Effect of pH on lipase production

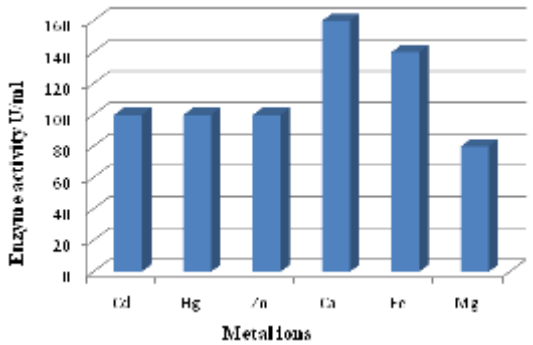

Fig. 5. Effect of metal ions on lipase production

respectively (Figure 1) after 120 hrs of incubation (Figure 2). Prolonged incubation did not affect the lipase yield, which might be related to the depletion of the nutrients or denaturation of the enzyme caused by the interaction with other components in the medium or change in pH of the medium.[11]

Temperature and pH are the most significant factors affecting the enzyme yield. With an increase in temperature, the production of the enzyme increased upto 37°C giving maximum lipase activity of 140U/ml. At temperatures above 37°C a rapid reduction in enzyme production was observed. This may be due to inactivation of the enzyme at higher temperatures (Figure 3). Each microorganism possesses a unique optimum pH and pH range for its growth and activity. It was observed that, the production of enzyme increases upto pH 8.0, with a maximum enzyme yield of 120 U/ml (Figure 4). With further increase in pH, a rapid reduction in enzyme production was observed.

Metal ions act as inhibitors as well as inducers of the enzyme, their presence or absence in turn can affect the yield of enzyme. Metals like Ca^{2+}, Cd $^{2+}$, Fe $^{3+}$ are known to act as inducers whereas Zn^{2+}, Mg^{2+}, Hg $^{2+}$ are known as inhibitors of lipase.[9] Ca^{2+} and Fe^{3+} ions were found to enhance the production of lipase giving an activity of 160U/ml and 140U/ml respectively. Mg^{2+} reduced the enzyme activity to 80 U/ml (Figure 5).

Thus the optimum conditions for large scale production of lipase by *Staphylococcus pasteuri* were found to be using YPDB containing 1% olive oil at after 120 hrs of incubation at a temperature of 37°C & pH 8. Metal ions like Ca^{2+} and Fe^{3+} enhanced the production of lipase.

CONCLUSION

Lipases have emerged as key enzymes in growing biotechnology, due to their multiple properties, which find wide applications in food industry, detergents, chemical industry and biomedical sciences.[12] Looking into the wide application of lipases the demand for lipase has

increased but, the production cost limits the industrial use due to which aiming for maximum lipase production is of interest for both academic & industrial purposes.[13] Increasing demands of lipases has led to the commercialization of lipase production and the search for new enzyme sources.

Optimization of parameters for large scale production of lipase enzyme was studied using *Staphylococcus pasteuri.* It was observed that it gave maximum lipase production after 5 days of incubation using olive oil as the substrate at a temperature of 37°C & pH 8. Metal ions like Ca^{2+} and Fe^{3+} enhanced the production of lipase. This study can be exploited for large scale production of enzyme which can be used in future to study the wide range of applications of the enzyme.

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