STUDIES ON PRODUCTION AND CHARACTERIZATION OF ALKALINE LIPASE FROM BACTERIA AND APPLICATION IN DETERGENT

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Abstract: Lipases are hydrolyses that act on carboxylic ester bonds and catalyse esterification, interesterification, acidolysis, alcoholysis and aminolysis in addition to the hydrolytic activity on triglycerides. These make them most versatile biocatalyst. They are of use in detergent, paper and pharmaceuticals industries. Commercial production of lipases from plant and animal sources is not economical and hence microbial lipases are increasingly important and they are diverse with respect to environmental conditions required for their activity. In the present work alkaline lipase producing bacteria namely A and B were isolated from soil sample. Isolate A and B showed respectively 72 hrs and 24 hrs optimum fermentation time period for production in tributyrene broth. The optimum pH and temperature for the activity of lipase of isolate A is 9.0 and 28^{o}C while of the isolate B is 8.0 and 37^{o}C respectively. Alkaline lipases of both the isolates could de-stain the oil stained cloths to a considerable extent and thus can be suitable as detergent additive.

Keywords: Alkaline lipases, lipase in detergent, pH, temperature.

Introduction Enzymes are natural catalysts, which permit endogenous biological reactions to occur rapidly through well defined pathway. They accelerate the rate of reaction, without being lost in the process. Enzymatic reactions occur within a narrow range of temperature and pH. Any change occurring in these crucial factors may lead to loss of structural integrity and complete deactivation of the enzyme. Lipases are part of the family of hydrolyses that act on carboxylic ester bonds. Their physiological role is to hydrolyze triglycerides to diglycerides, monoglycerides, fatty acids and glycerol. They usually exhibit good chemoselectivity, regioselectivity and enantioselectivity. Lipases posses broad substrate specificity and can be found with optimum activities over a wide range of temperature. These are versatile catalyst and can be used in a number of biotechnological applications involving hydrolysis, synthesis or exchange of ester bonds **[13].** Lipases are widely distributed in animals, plants and microorganisms **[11].** Currently, commercialized lipases are produced by fungi, yeast and bacteria due to the facility to cultivate organisms on a large scale pancreatic lipase is the best known and the most often investigated lipolytic enzyme. Bacterial lipases from the genus *Pseudomonas* exhibit the most versatility, reactivity and stability in catalyzing reactions in a nonaqueous environment **[5].**

Fungal lipases are being exploited due to their low cost of extraction, thermal and pH stability, substrate specificity and activity in organic solvent. The chief producers of commercial lipases are *Aspergillus niger*, *Candida cylindracea, Mucor miehei.*

Because lipases are active in aqueous and nonaqueous solvent systems, it has become evident that lipases have considerable applications in industry and medicine **[15].** In this work an attempt was undertaken to isolate

bacteria producing lipases and studying the characteristics of their lipases, as well as their utility in detergents.

Materials and methods:

Collection of sample: - For isolation of lipase producing organisms, oil-tank scrap samples from Umbraj and oily black soil sample from Karad were collected in separate polythene bags and stored in refrigerator till further use. Both areas are from Karad tahsil, District Satara, Maharashtra.

Enrichment of sample: For enrichment of lipolytic bacteria, 1.0 gm of each sample was separately inoculated in 100 ml of Tributyrene broth of pH 9.0 and then flasks were incubated at 28^{O}C for 2 days on shaker.

Screening of alkaline lipase producing bacteria:

Alkaline lipase producing bacteria were isolated by inoculating enriched samples on sterile Tributyrene agar plate having pH 9.0 and incubating at 28^{O}C for 2 days. After incubation, colonies showing zones of hydrolysis around them were selected as lipase producing bacteria. The isolates were purified, subcultured and after proper labeling, preserved in refrigerators on Tributyrene agar slants till further use. Well isolated colonies were used for further studies.

The efficiency of isolate to produce lipase enzyme was determined in terms of selection ratio (S.R) on Tributyrene agar plates after incubation at 28^{o}C for 2 days. SR values were calculated as given below,

$$SR = \frac{\text{Diameter of zone of hydrolysis(mm)}}{\text{Diameter of colony (mm)}}$$

Isolates showing higher SR value were selected for further studies.

Production of alkaline lipase:

Submerged culture technique was used for enzyme production, wherein 200ml of sterile fermentation broth was inoculated with 10ml of inoculums prepared in sterile tributyrene broth from selected isolates separately. The flasks were incubated on shaker incubator at 28^{O}C for 4 days. After every 24hrs, 50ml broth was separated from production media and placed for centrifugation at 5000rpm for 15mins. After centrifugation supernatant containing crude enzyme was used for lipase assay. The results were used for determining the optimum time for the production of enzyme.

Assay of lipases: Copper soap colorimetric method **[10]** was used for the lipase assay. The enzyme was assayed in the form of amount of free palmitate produced from groundnut oil.

Characterization of alkaline lipases:

The enzyme was characterized for studying effect of temperature, pH, substrate concentration and metal ions on it.

- Effect of pH on alkaline lipase activity was studied by running enzyme reaction at different pH values such as 2,3,4,5,6,7,8,9,10&11, and then assaying enzyme in it.
- Effect of temprature on alkaline lipase activity was studied by allowing enzyme to act at different temprature values such as 10^{O}C, 28^{O}C, 37^{O}C, 45^{O}C, 55^{O}C, 75^{O}C and 100^{O}C. The enzyme reaction was run at pH8 for isolate B and pH9 for isolate A.

Temperature and pH values showing higher units of enzyme were noted as optimum temperature and pH for lipase activities.

- Effect of substrate concentration was studied by using different concentration of groundnut oil viz.0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0ml as substrate for assay system. Graph of substrate concentration v/s absorbance at 715nm was plotted and effect of substrate concentration was studied.

Suitability of alkaline lipase enzyme as detergent additive:

The enzyme was applied on the groundnut and coconut oil stained cotton and terry-cot fabrics. Two different oils namely, groundnut and coconut oils mixed with Sudan black-B stain were used for staining cotton and terry-cot fabrics. The stained fabrics were treated with only distilled water and water plus detergent as control, with only enzyme and with enzyme plus detergetnt combination as tests. The mixtures were incubated at 28^{O}C for 30mins, and then the fabrics were washed under tap water. Then the decolourisations were observed visually and rated from 1 to 5 for increasing decolourisation.

Results and Discussions: Two bacterial isolates namely, A and B producing alkaline lipase were obtained from soil sample, while the isolate C was obtained from Oil tank scrapping. Isolate A and C were characterized as Gram positive cocci and isolate B as Gram negative rod.

SR values given by the isolates A, B and C were 8, 5 and 1.6 respectively, indicating that the isolate A could produce maximum lipase while C could produce minimum. So the isolates A and B were used in further studies. Results of day wise production of lipase by the isolates were as shown in Figure No.

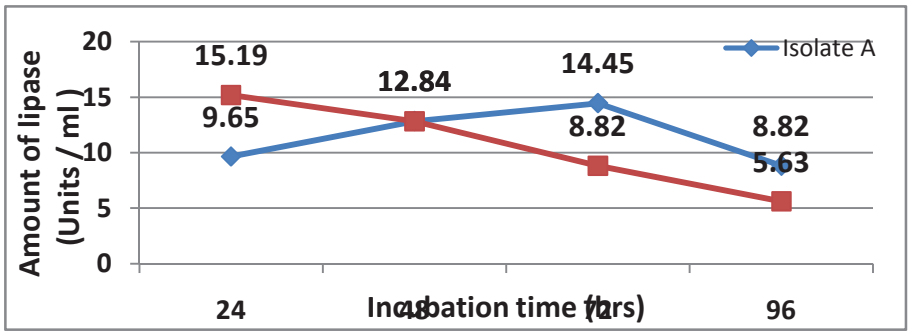

Figure No.1: Amount of enzyme produced day wise by the isolates

Table No 1. Effect of incubation time on the units of enzyme produced by the isolates

Isolate	Units of enzyme produced with Incubation time (hrs)			
	24	48	72	96
A	9.6	12.84	14.45	8.82
B	15.19	12.84	8.82	5.63

It can be seen from the figure No.1 that the isolate A showed increase in the amount of enzyme activity with increase in the fermentation time up to 72 hrs. Isolate A produced maximum enzyme at 72 hrs of incubation. Subhajit (2012) also found that lipase of *Serratia* spp was produced maximum in 72 hrs. While the isolate B produced maximum enzyme at 24 hrs of incubation. The amount of enzyme produced by isolate B got decreased with increase in the time of incubation.

Results of effect of pH on enzyme activity were as shown in figure No. 2 and table No 2.

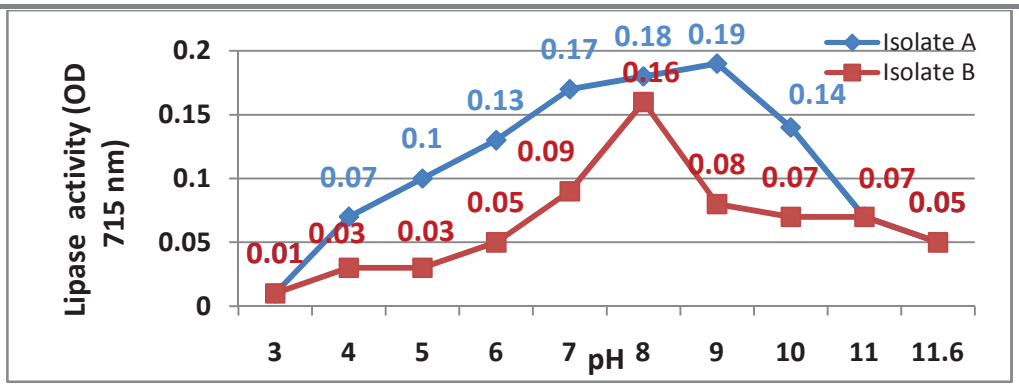

Figure No.2: Effect of pH on enzyme activity

Table No.2: Effect of pH on enzyme activity expressed as OD 715										
Isolate	Enzyme activity (OD_{715}) at pH									
	3	4	5	6	7	8	9	10	11	11.6
A	0.01	0.07	0.1	0.13	0.17	0.18	0.19	0.14	0.07	0.05
B	0.01	0.03	0.03	0.05	0.09	0.16	0.08	0.07	0.07	0.05

It can be seen from the figure No. 2 and table No. 2 that lipases of both the isolates showed a typical bell shaped response to pH with maximum activity expectedly in the alkaline range which was between 8 and 9. Optimum pH for activity of lipase of isolate A was found to be 9.0 while for B it was 8.0. Although for *Aspergillus*, alkaline lipase studied by Jin-Ian Xia et.al. (2011) was found functioning optimum at 8.5 pH. Lipase of *Staphylococcus* was found optimally acting at pH 6.5, (Pavia et.al., 2000), Optimum pH for the lipase of *Bacillus* spp RSJ1 was found to be 8.0 by Sharma et.al (2001).

Results of effect of temperature on enzyme activity were as shown in table no 3 and figure No. 3

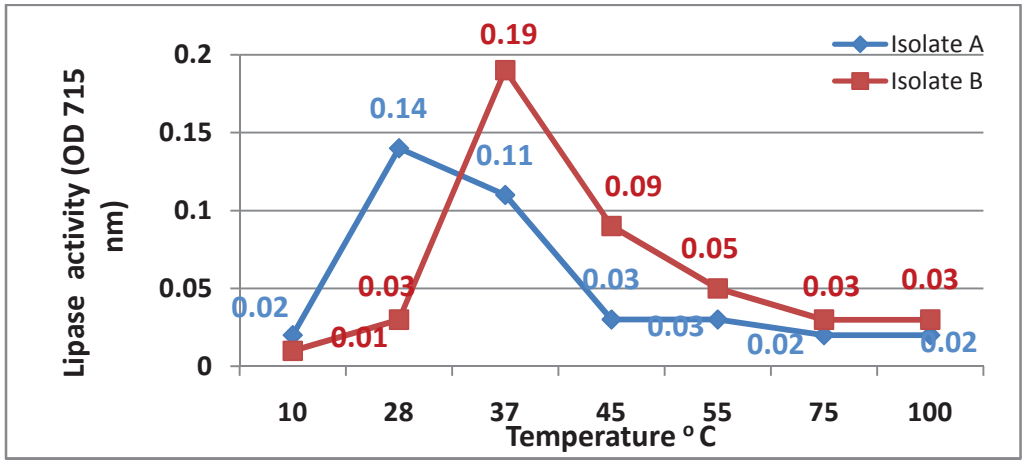

Figure No.3: Effect of temperature on enzyme activity

Table No.3: Effect of temperature on enzyme activity expressed as OD(715)							
Isolate	Enzyme activity (OD_{715}) at temperature (^{o}C)						
	10	28	37	45	55	75	100
A	0.02	0.14	0.11	0.03	0.03	0.02	0.02
B	0.01	0.03	0.19	0.09	0.05	0.03	0.03

Figure No 3 shows that lipase activity of the isolate A at various temperatures was ranging from 0.02 to 0.14. The activity was found increased with increase in the temperature up to $28^{O}C$. which got reduced further above it. The lipase of the isolate B showed maximum activity at $37^{O}C$ which was found reduced below as well above it. These results indicate that the lipases of the isolate A and B showed their maximum enzyme activity at $28^{O}C$ and at $37^{O}C$ respectively. Gunsekharan (2006) found $40^{o}C$ as an optimum temperature for activity of lipase of *Citrobacter* spp.

Results of effect of substrate concentration on enzyme activity were as shown in figure No. 4

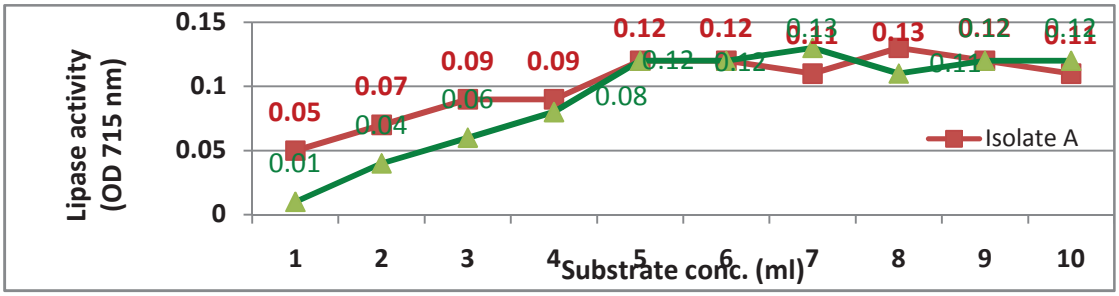

Figure No.4: Effect of substrate concentration on enzyme activity

The results in figure No. 4 show that for both the isolates, as substrate concentration increased. Enzyme activity also increased upto 0.5 ml, beyond which enzyme activity remained constant. The Km and Vmax values were determined from graph. The Km values for the enzyme of the isolate A and B were 0.15, while Vmax values for both isolates was 0.12.

Destaining results showed that both fabrics were destained in only distilled water but to very little extent which was rated as 1. In comparison to this the destaining by only lipase was rated as 2 for both the lipases. The enzymes destained better with detergents which was rated as 4 and 5 by the lipase of the isolates A and B respectively. It was observed that when the lipase was added with any detergent on any type of fabric, greater amount of all kinds of oil due were removed than that in case of the detergent alone. Alkaline lipase of *Pseudomonas* spp have been found by Kanimozhi and Perinbam (2011) to be of great use as detergent additive.

Conclusion: Isolate A and B were potent producers of alkaline lipase. Although isolate 'A' took 74 hrs time for maximum production of lipase, 'B' could produce maximum amount of lipase within 24 hrs. It could save the time of enzyme production. Optimum pH for the activity of lipase of isolate A was 9 and that for B was 8. This property of lipase shows that they can be of use in alkaline environment. Optimum temperature for the activity of lipase of isolate A was 28^{O} and for B was $37^{O}C$. This indicates that the enzyme of isolate 'B' could act in warm

condition as well. The Km values for the enzyme of the isolate A and B were 0.15, While Vmax values for both isolates was 0.12. Alkaline lipase of both isolates could destain the oil stained cloths to a considerable extent It showed that these lipases have the potential for applications in detergent industry.

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