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Original Article

CHARACTERIZATION OF PARTIALLY PURIFIED LIPASE FROM SACCHAROMYCES CEREVISIAE

KANDERI DILEEP KUMAR[1], K. Y. USHA[1], SATYANARAYANA S. V.[2], SAILAJA V.[3]

[1]Department of Microbiology,Sri Krishnadevaraya University,Anantapuramu 515003, A.P, [2]Departments of Genomic Sciences, Central University of Kerala, Kasaragod 671328, Kerala, [3]Department of Zoology, Vikrama Simhapuri University Post Graduate Centre, Kavali, Nellore 524201, A.P. India
Email: kanderidileepkumar@gmail.com

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ABSTRACT

Objectives: This study was aimed for the purification and characterization of lipase from *Saccharomyces cerevisiae* and its activity.

Methods: Extracellular lipase enzyme was extracted from the culture filtrate of baker's yeast *Saccharomyces cervisiae* grown on lipase production medium. The enzyme was purified by ammonium sulphate fractionation attaining maximum activity at 75% w/v saturation. The dialyzed sample was then subjected to sephadex G 100 column chromatography. The enzyme was purified 4.1 fold with a specific activity of 30 U/mg.

Results: The molecular mass of this enzyme was estimated to be approximately 46 kDa by SDS-PAGE. The enzyme was stable even after exposure to a pH range of 4.0-8.0 and at 45^{o}Ctemperature for 1 hour. The enzyme exhibited optimum activity at pH 5.0-6.0 and at temperature 30^{o}C. Its activity was greatly enhanced by the addition of 10 mM 2- mercaptoethanol and completely inhibited by Hg^{+2} indicating that the enzyme contains sulfhydryl group at its active site.

Conclusion: The significance of controlling parameters for lipase activity, and it's the effect on different parameters like temperature, P^{H} and metal ions on partially purified lipase enzyme having molecular weight of approximately 46 kDa.

Keywords: Ammonium sulphate, Baker's yeast, Gel filtration, Lipase, SDS-PAGE, Metal ions.

INTRODUCTION

Lipases (Triacylglycerol acylhydrolases, E.C.3.1.1.3) are ubiquitous water-soluble enzymes of considerable physiological significance and industrial potential. Lipases catalyze the hydrolysis of triacylglycerols to release free fatty acids and glycerol. A true lipase will split emulsified esters of glycerol and long-chain fatty acids such as triolein and tripalmitin. Lipases are serine hydrolases and have immense biotechnological applications. Lipases are a particular group of enzymes with many applications such as in textile, food, biomedical, petrochemical, pharmaceutical, detergent, and many other industries [1]. Besides their industrial applications, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers, biodiesel, production of enantiopure pharmaceuticals, agrochemicals and flavour compounds [2].

Lipases are produced by both eukaryotes (including animals, plants and fungi) and prokaryotes, including bacteria and archaea [3]. Microbial lipases have received great attention due to a variety of their biochemical activities and ease of their isolation and production [4]. In eukaryotes, lipases are involved in various stages of lipid metabolism including fat digestion, absorption, reconstitution and lipoprotein metabolism [5].The lipases catalyze a wide range of reactions, including hydrolysis, inter-esterification, acidolysis, esterification and aminolysis. They catalyse the hydrolysis of fatty acid ester bond in the triacylglycerol and release free fatty acids [6].

Yeast has been used in food and other industries since ages. They have earned acceptability since long and are considered natural. Yeasts are also considered to be easy to handle and grow, in comparison with other bacteria [7]. Among microbial lipases extensive reviews are available on bacterial lipases [8, 9]. Yeast lipases have received a raw deal despite the fact that *Candida rugosa* is the most frequently used organism for lipase synthesis. [10] Reviewed exclusively lipase production by *Candida rugosa*. A Japanese company used *Candida rugosa* lipase for production of fatty acids from castor bean long back in 1985 [11]. The information on numerous other yeast lipases is scattered. The present study was aimed for purification and characterization of lipase from *Saccharomyces cerevisiae.*

MATERIALS AND METHODS

Baker's yeast (Saccharomyces cerevisiae) was obtained from Bakery and the culture was maintained at low temperature (i.e., nearly 4^{o}C) by periodic transfer on YEPD (Yeast Extract Peptone Dextrose) agar plate containing yeast extract (10g/l), peptone (20g/l), glucose (20g/l) and agar (20g/l). The production medium with coconut oil (tryptone-1%, yeast extract-0.5%, NaCl-0.5%, supplemented with $CaCl_2.2H_2O$-0.01%, tween 80-1%) was prepared. The yeast culture was inoculated into enzyme production medium. The culture was harvested, filtered, and centrifuged. The supernatant was used for further enzyme assay and lipase purification.

Purification of Extracellular lipase of *Saccharomyces cervisiae*

Lipase was purified from the growth medium after the removal of cells by centrifugation. The enzyme was purified by ammonium sulphate precipitation and Sephadex G-100 size-exclusion chromatography. Prior to purification studies, optimum ammonium sulphate concentration for the precipitation of lipase was determined by ammonium sulphate fractionation. Appropriate amount of ammonium sulphate was added to the supernatant and stored overnight at room temperature, centrifuged at 12000 rpm for 1hr to collect the precipitated ammonium sulphate and protein complex. This precipitate was dissolved in 100 mM Tris phosphate buffer (pH 7.5) and subjected to dialysis to get pure protein free from ammonium sulphate. The protein pellet obtained after dialysis was loaded onto a Sephadex G-100 column (2 × 40 cm) and was eluted with a 50 mM sodium acetate buffer (pH 5.0) at a flow rate of 15 ml/h. The fractions of 3 ml were collected and the absorbance and lipase activity were measured. Further SDS-PAGE electrophoresis was carried out to determine molecular weight of lipase [12]. In the present study, 12% resolving gel and 4% stacking gel were used to separate the proteins.

Characterization of Purified Enzyme

Effect of pH and temperature on lipase activity and stability

The pH effect was determined by incubating the purified lipase (10 µg as protein) at different pH levels (4-11) under standard assay conditions using colloidal chitin as the substrate. Acetate buffer (50

mM) was used for pH 3-6, phosphate buffer (50 mM) for pH 7, Tris-HCl buffer (50 mM) for pH 8-9, and glycine-NaOH buffer for pH 10-11. The enzyme stability was determined after preincubation at various pH levels without the substrate for 16 h.

Effect of Temperature on Enzyme Activity and Stability

The optimum temperature for the lipase activity was determined by performing the standard assay in the range of 20-80°C. Thermal stability was determined by assaying the residual lipase activity after incubation for 1 hr at the previous temperature without the substrate.

Effect of Various Metal Ions on Lipase Activity

The enzyme was preincubated with a 10 mM concentration of different metals and inhibitors. After 30 min, the remaining lipase activity was measured using the standard assay. The relative inhibition of the calculated enzyme was based on the release of N-acetyl glucosamine.

Lipase assay

To 250 mg of olive oil 2 ml of phosphate buffer (pH 6.3) was added in a test tube. 1 ml of enzyme sample was added and vortexed for 15 sec. The mixture was incubated at 37°C in a water bath under static conditions for 30 min. 1 ml of concentrated Hcl was added and vortexed for 10 minutes. Then, 3 ml of benzene was added and vortexed for 90 sec. From this 2 ml of benzene layer was taken and added to 1 ml aqueous solution of 5 per cent cupric acetate (pH 6.2). Then it was vortexed for 90 sec and centrifuged at 5000 rpm for 10 min. A clear organic phase of benzene layer was removed and used for the estimation of liberated fatty acids by measuring the optical density at 715 nm.

RESULTS AND DISCUSSION

Lipase production media of Yeast culture

One percent of inoculum was taken from the selective medium (LIPASE MEDIUM) and added to the culture. 0.319 O.D was observed at 715 nm and by this it is confirmed that lipase enzyme activity was present.

Ammonium sulphate precipitation

Ammonium sulphate precipitation of crude sample or supernatant showed maximum activity at 75% saturation. The precipitated protein was dissolved in acetate buffer, pH 5 and this protein was subjected to dialysis and dialyzed protein is used for further studies.

Sephadex G-100 Gel Filtration Chromatography

The protein obtained from dialysis was loaded onto a column of Sephadex G-100 (2× 40 cm) equilibrated with acetate buffer, pH 5. The elution profile of gel filtration chromatography is shown in Figure 1

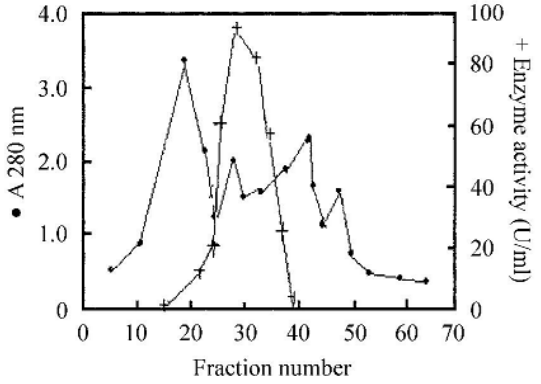

Fig. 1: Elution profile of Yeast lipase by Sephadex G-100 column

From the elution profile, it was observed that the lipase activity was maximum at fraction-30 (15-39). Maximum lipase activity fractions were pooled, dialyzed, and concentrated by lyophilisation and used for further studies. The summary of purification steps involved for lipase is presented in Table 1.

Table 1: Summary of Purification Steps of Lipase from *Saccharomyces cervisiae*

Purification method	Total protein (mg)	Total activity (Unit)	Specific activity (U/mg)	Purification fold
Culture filtrate	425	3078	7.24	1
Ammonium sulphate precipitation	220	3,130	14.2	1.9
Sephadex G-100	42	1,200	30	4.1

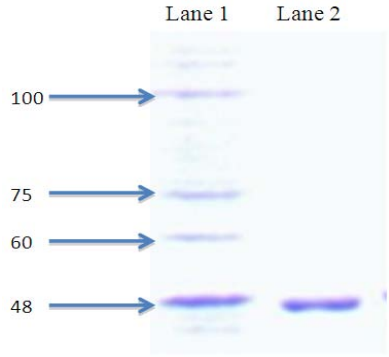

Fig. 2: SDS-PAGE showing the purified lipase, Lane 1: Bio-Rad high range protein marker , Lane 2: Purified lipase (46kda)SDS-PAGE of Purified Lipase from *Saccharomyces cervisiae*

The purified lipase was analyzed by SDS-PAGE. The purified lipase showed a single band, indicating a homogeneous preparation. The molecular weight of the lipase was determined by comparison of the migration distances of standard marker proteins and interpolation from a linear semi logarithmic plot of relative molecular mass versus the R_f value (relative mobility). Depending on the relative mobility, the molecular weight of the protein band was calculated to be **46 kDa** (Fig.2). The molecular weight of two forms of lipases isolated from *Geotrichum candidum* ATCC 34614 was estimated to be 56 kDa [13]. An extracellular lipase produced the glycolipid producing yeast *Kurtzmanomyces sp I-11* had a molecular weight of about 49 kDa as estimated by SDS-PAGE [14].

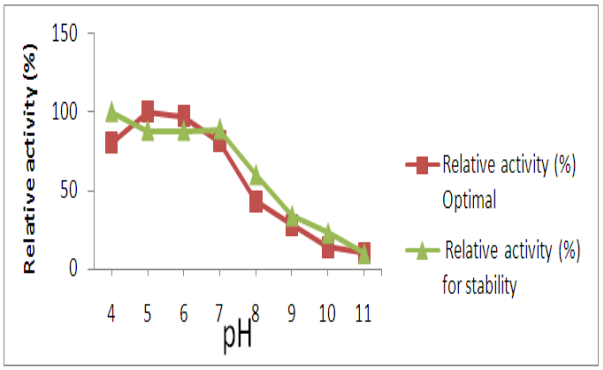

Fig. 3: Effect of pH on lipase activity

Characterization of Purified Enzyme

Optimum pH and pH Stability

The optimal pH for lipase activity and stability of the lipase were examined. The enzyme was most active between pH 5.0 and 6.0. It was relatively stable at pH between 4.0 and 8.0, when kept at 4°C. However, beyond these pH ranges, it rapidly lost its activity (Fig. 3). Many lipases, including the present one, showed a pH optimum in the acidic range. The three forms of lipases produced by *Candida rugosa* in solid state fermentation showed optimal activity at pH 7-8 [15].The stable pH range of *Trichosporon fermentans WU-C12* was 4-8 [16].while that of *Alteromonas* sp. Strain O-7 [17] was in the basic range and *Bacillus thermoleovorans ID-1*was in the neutral range [18].

Optimum Temperature and Thermal Stability

When the enzyme was incubated at various temperatures for 1 h in an acetate buffer (pH 5.0), it was highly stable at temperatures below 40°C and it retained almost half of its activity at temparature50°C and only 15% of residual activity was noted at 60°C the lipase activity was most active at 30°C. (Fig.4).Similarly Boer et al. [19] also reported that the optimum temperature for lipase from *Arxula adeninivorans*is 30°C.Bacterial lipases generally have temperature optima in the range of 30 to 60°C [20].

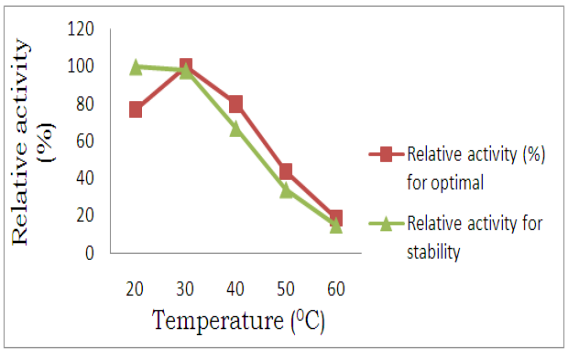

Fig. 4: Effect of temperature on lipase activity

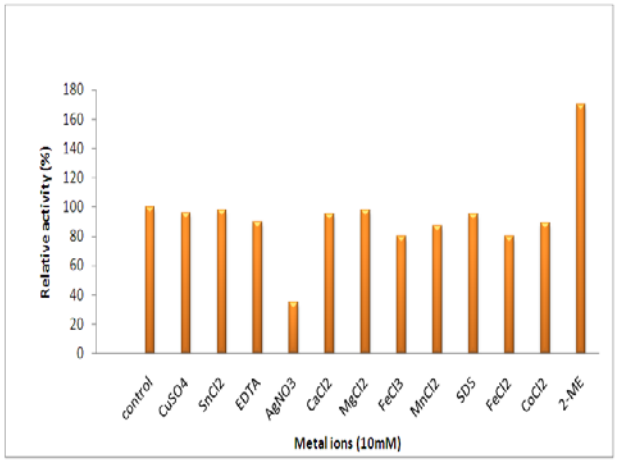

Fig. 5: Effect of various metal ions on extracellular lipase activity from *Saccharomyces cervisiae*

Effect of Metal Ions and Chemical reagents

Metal ions are responsible for the maintenance of the stability of lipases [21]. Thus to find out whether the different metal ions stabilize or destabilize the enzyme, the effect of different metal ions and a metal chelator EDTA at 10 mM concentration on lipase activity was investigated and results are summarized in fig 5. In the present study control sample which did not contain any additives was used and it is assumed as hundred percent while calculating relative enzyme activities. As shown in fig 5, the lipase activity was strongly inhibited by Ag+ and completely inhibited by Hg+. Most of metal ions had a negative effect on the lipase activity. Generally, lipases are not sulfhydryl proteins; and thus in most lipases neither free –SH nor S–S bridges are important for their catalytic activity. This is substantiated by the use of 2-mercaptoethanol, p-chloromercuric benzoate and iodoacetate, which have no detectable effect on lipase from *C.viscosum* [22]. But in the present study the increase in activity with 2-mercaptoethanol indicated the presence of sulfhydryl groups on the active site of the enzyme. Similar inhibition and 2-mercaptoethanol enhancement was reported by Ueno *et al.* [23]. However not much decrease in the lipase activity was observed in the presence of all the metal ions incubated. Except for Ag^{+} above 80% of the activity was retained in all the metal ions. Lipases from thermophilic *Rhizopusoryzae* and *Mucor sp.* isolated from palm fruit showed very depressed activity in the presence of $Hg^{2+}$with respect to our lipases [24, 25].

CONCLUSION

The present study demonstrated the importance of controlling parameters for lipase activity, and assesses the effect of different parameters like temperature, pH and metal ions on partially purified lipase enzyme having molecular weight approximately 46 kDa. The enhanced activity of lipase in the presence of 2-mercaptoethanol indicates that it contains sulfhydryl groups at its active site in contrast to other most studied lipases.

CONFLICT OF INTERESTS

Declared None

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