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RESEARCH ARTICLE

KINETIC STUDIES OF PARTIALLY PURIFIED LIPASE FROM MARINE ACTINOMYCETE *STREPTOMYCES ACRIMYCINI* NGP 1

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ABSTRACT

This study was focused on partial purification and characterization of lipase from *Streptomyces acrimycini* NGP 1, isolated from marine sediment of south Indian coastal region. In purification steps, 4.53 fold purification was achieved after 85% ammonium sulphate precipitation with 0.97 percent recovery. In further purification steps, 1.33 fold purification was achieved by Sephadex G-100 chromatography with 1.61 percent of recovery. The specific activity of purified enzyme was 1525 U/mg. Zymogram of crude enzyme on native-PAGE presented bands with lipase activity of molecular weight and Isoelectric point were 50 kDa and 7.4 respectively. These features render this lipase of interest as a biocatalyst for applications such as biodiesel production and detergent formulations.

Keywords: Lipase, Marine sediment, Purification, Application

1. INTRODUCTION

Lipases are serine hydrolases characterized as triacylglycerol acylhydrolases (E.C. 3.1.1.3) and ought to be separated from esterases (E.C. 3.1.1.1) by the idea of their substrate. In reality, a criteria used to recognize these two kinds of catalysts, i.e., actuation by the nearness of an interface, additionally called "interfacial initiation," was discovered unacceptable for the arrangement of such proteins as a few lipases didn't show such marvel. Prominent cases of this phenomenon are Lip4 from *Candida rugosa* (Barriuso *et al*., 2016) and *Candida antarctica* B (Zisis *et al*., 2016). In addition, lipase and esterase accord themes portrayed by PROSITE database (Hulo *et al*., 2006). In addition, lipases are capable of hydrolyzing water-insoluble esters and show a different distribution on the hydrophobic amino acids surrounding the active site (Chahinian and Sarda, 2009). Lipases, in thermodynamic good conditions (i.e., low water movement), likewise catalyze an enormous assortment of blend responses which can be grouped into two principle kinds of responses, i.e., esterification what's more, transesterification (Kapoor and Gupta, 2009).

First lipases were isolated by Eijkman from Bacillus prodigiosus, Bacillus pyocyaneus, and *Bacillus fluorescens*, currently known as *Serratia marcescens*, *Pseudomonas aeruginosa*, and *P. fluorescens*, respectively (Eijkmann, 1901). Lipase are produced by various organisms, including animals, plants and microorganisms. Most animal lipases are obtained from the pancreas of cattle, sheep, hogs, and pigs. Unfortunately, lipases extracted from animal pancreas are rarely pure enough to be used in the food industry (Sharma and Kanwar, 2014). Because of their capacity to utilize fat as the main carbon source, microorganisms delivering lipases have been separated from nourishment waste, where they are answerable for the enhance change of dairy items, for example, cheddar, or from slick situations (sewage, waste dump locales, and oil plant emanating) (Li *et al*., 2014). Due to the importance and wide variety of lipase applications, different techniques have been developed in order to isolate lipases from various sources. Lipase enzyme was purified by sequential methods of ammonium sulphate precipitation and Sephadex G-100 gel column chromatography (Tripathi *et al*., 2014). Further the molecular weight of the enzyme was analyzed and kinetic studies were carried out.

The current study presents sequential optimization strategy to improve lipase production using *S. acrimycini* NGP 1. Partial purification and characterization of the partially purified enzyme were investigated and these parameters are necessary to use the enzyme efficiently in an industrial application.

2. MATERIALS AND METHODS

2.1. Isolation of Actinomycete

The marine sediment was collected from the coastal region of Marina, Tamilnadu, India at 2-3m depth by using grab sampler. The collected

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sediment was subjected for enrichment prior to serial dilution. One gram of enriched sample serially diluted using sterile distilled water and spread over starch casein Agar (SCA) plates and incubated at 28 + 2^{O}C for 7 days (Balagurunathan and Radhakrishnan, 2009).

2.2. Lipase Screening

The marine actinomycete isolate was subjected for rapid screening of lipase production by rapid plate method (Kouker and Jaeger, 1987). The screening of the actinomycete isolates for lipase activity was studied by inoculating them on Rhodomine B medium. Colonies which showed orange fluorescence under UV irradiation indicated lipase activity and was measured by Noomrio *et al*, (1990). The amount of liberated fattyacid was determined by titration with 0.05M NaOH. The enzyme activity was expressed as U/ml (IU = release 1 µ mole of fattyacid in 1 min).

2.3. Genetic Identification

The genetic level identification of potential actinomycete isolates were carried out. Phylogenetic relationships with closely related species were determined by using MEGA version 4.0. Distance matrices were determined and were used to elaborate a dendrogram by the neighbor-joining method (Tamura *et al*., 2007; Teske *et al*., 1996).

2.4. Growth Kinetics and Enzyme Production

To determine the optimum culture conditions for enzyme production, the actinomycete isolates were grown in basal liquid medium in an orbital shaker at 120 rpm.

2.5. Partial Purification of lipase enzyme

The purification procedure was referred to the method of Shu *et al* , (2006). The culture filtrate was collected through whatmann No. 1 filter paper and the filtrate was centrifuged at 4000 rpm for 10 min to obtain crude enzyme. Then the ammonium sulphate was added to the filtrate to give final concentration of 60.0 - 80.0 per cent (w/v) saturation at 4ºC. The precipitate was collected by centrifugation at 4000 rpm for 4 min. The precipitate was then dissolved in 20 ml of double distilled water and dialyzed for 24 hrs at 4ºC against double distilled water and concentrated by lyophilization.

An aliquot of the lyophilized sample (1 ml) was loaded on to a sephadex G-100 column (45 X 1.5 cm) previously equilibrated with 0.02 M phosphate buffer (pH 7.8). Elution of lipase was performed by a linear gradient of NaCl (0-1.0 M/l in equilibrating buffer) at 30 ml/h. The fractions of 5.0 ml each were collected for every one hour (30 fractions) and assayed for lipase and protein activity.

2.6. Optimum Substrate Concentration

The optimum substrate concentration for the maximum activity of the enzyme determined in terms of maximum reaction velocity (V max) and michaelis constant (Km at which reaction velocity is half maximum). For this, various concentrations of specific substrates were prepared and incubated with purified enzyme preparations. For Lipase activity, 1 to 10 mM of olive oil in acetate buffer (20 mM; pH 4.0) was used as substrate (Zhou *et al*., 2012). V max and Km were estimated graphically by plotting substrate concentration in µM on X axis against enzyme activity U/mg protein on the Y axis. The accurate values of Vmax and Km were obtained by double reciprocal Line Weaver-Burk plot and Eadie-Hofstee plot. The protein content was estimated and the molecular weight of the sample was determined by SDS-PAGE (Zouaoui and Bouziane, 2012).

2.7. Determination of Pi Values

The pI values of each enzyme fraction were determined by Iso electric focusing (IEF) technique (Zouaoui and Bouziane, 2012).

3. RESULTS AND DISCUSSION

3.1. Screening and Identification

The actinomycetes growth occured on the SCA plate was subjected for Lipase screening in the Rhodomine B agar medium and it produced maximum enzyme of about 40 U/ml. The genetic level analysis of the 16S rRNA gene is the most important tool for correct identification of microbial species. The isolate was identified as *S. acrimycini* NGP 1 and the sequence was submitted to Gen-Bank (JX843532). A phylogenetic tree constructed by MEGA 4 software based on 16srRNA partial sequence. Similarly, 16SrRNA phylogenetic analysis of actinomycetes isolated from Eastern Ghats was carried out neighbor-joining algorithm (Nithya *et al*., 2012). Similarly, 55 samples from different regions were selected and screened by Rhodamine B flat transparent circle method to observe lipase producing effect, among them, Serratia sp. has the characteristics of fast growth, high enzyme production and stable ability (Gupta *et al*., 2013)

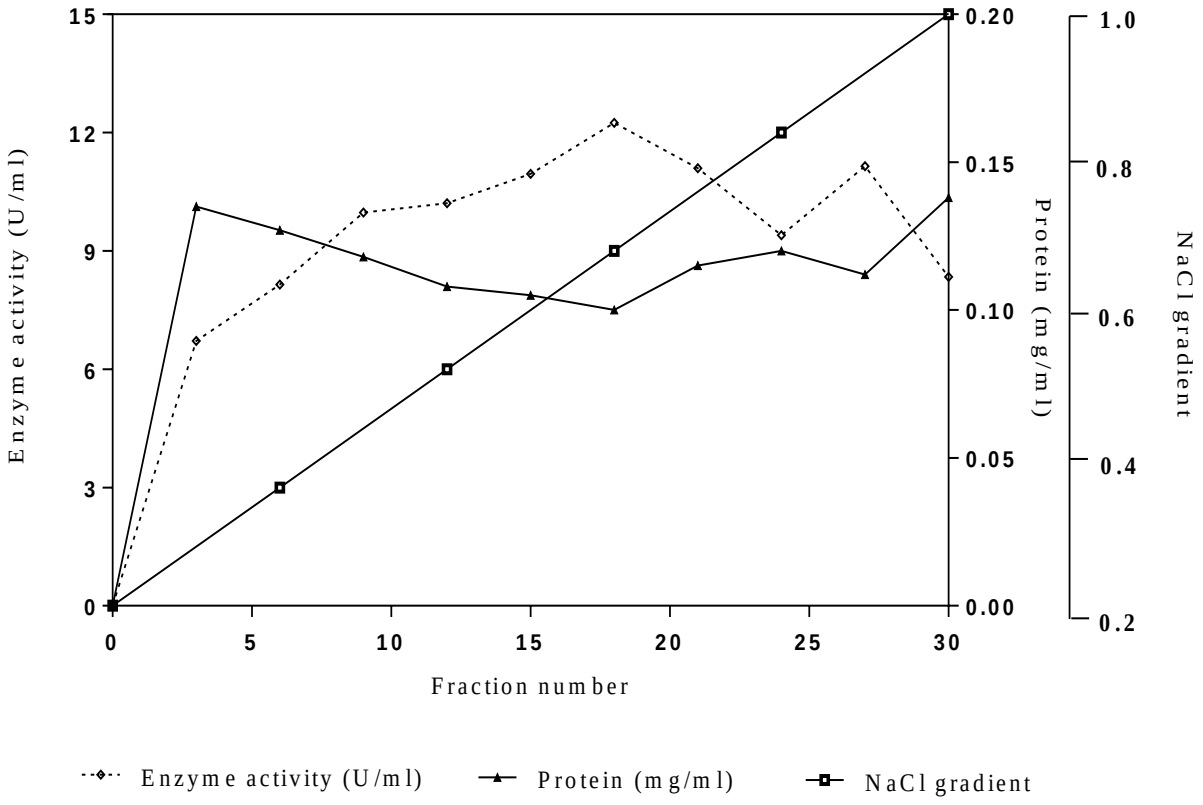
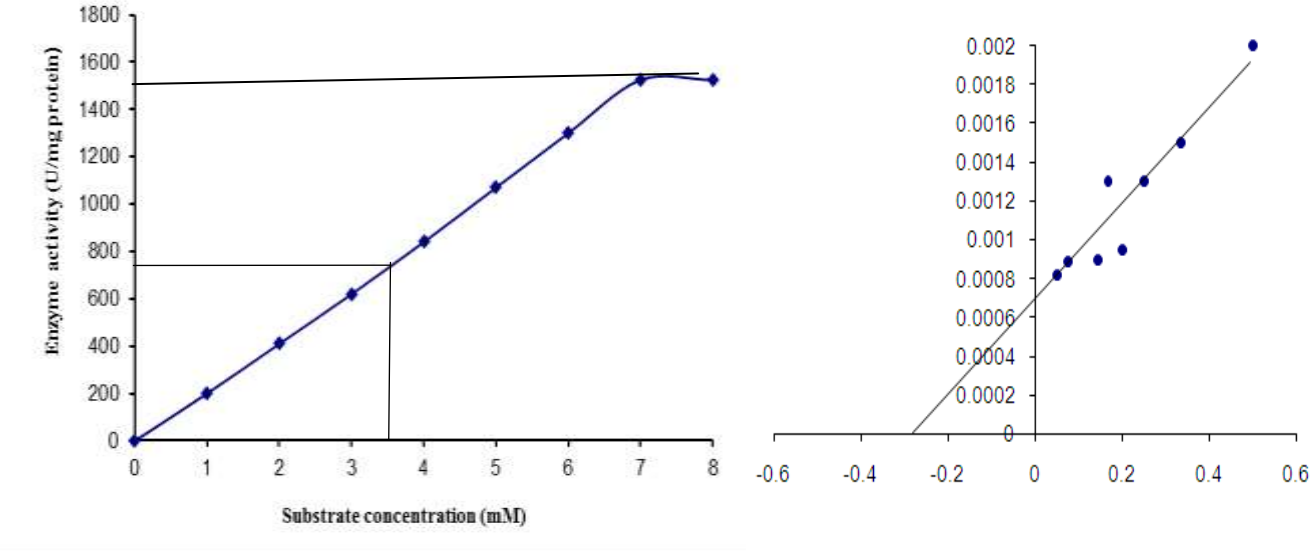


Fig 1: Purification of lipase from *S. acrimycini* NGP 1 on Sephadex G 100 column

Table 1. Purification of lipase from the culture filtrates of *S. acrimycini* NGP 1

Sample	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Total Activity (U)	Specific Activity (U/mg)	Recovery Yield (%)	Purification factor
***Streptomyces acrimycini* NGP 1**							
Culture filtrate	500	9.15	0.12	4575	76.25	100	1.00
Ammonium sulphate precipitation	20	10.37	0.14	207.4	74.07	4.53	0.97
Column Chromatography Sephadex G - 100							
Fraction 18	5	12.25	0.10	61.25	122.5	1.33	1.61

V max **Double reciprocal plot**



Eadie-Hofstee plot

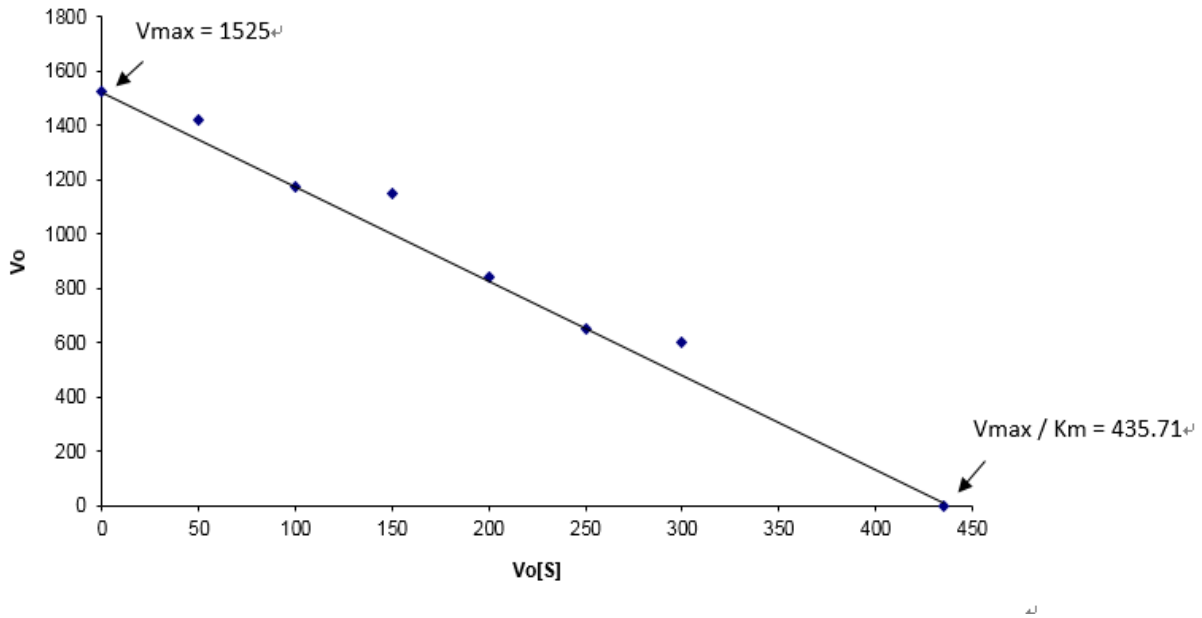


Fig. 2: Kinetics of *S. acrimycini* NGP 1 lipase

3.2. Growth kinetics and Enzyme production

The results showed that the culture filtrate of *S. acrimycini* NGP 1 had lipase total activity of 9.15 U/ml with a protein content of 0.12 mg/ml; the specific activity was 76.25 U/mg protein. When concentrated by ammonium sulphate, the specific activity was decreased to 74.07 U/mg protein with a purification factor of 0.97. The protein content was increased to 0.14 mg/ml. The enzyme recovery was 4.53 per cent. When passed through sephadex G 100 column, the fraction 18 exhibited lipase activity. In fraction 18, the protein content was 0.10 mg/ml; specific activity was 122.5 U/mg proteins, the purification fold and recovery yield were 1.61 and 1.33 per cent (Fig. 1 and Table 1). Similarly, lipase enzyme from *Microbacterium sp*. was partially purified by ammonium sulphate and Sephadex G100 column chromatography. This purification procedure resulted in 2.1fold purification of lipase with a 20.8 % final yield (Tripathi *et al*., 2014)

The kinetic properties of lipase were obtained using 1 to 10 mM of olive oil as substrate in a Lineweaver-Burk plot. In lipase, Vmax and Km values were estimated by using olive oil in acetate buffer (20 mM; pH 4.0); Vmax and Km for *S. acrimycini* NGP 1 lipase were 1525 U/mg protein and 3.5 mM respectively (Fig. 2). The V_{max} of the purified lipase from a newly isolated *Trichosporon coremiiforme* strain was 1800 U/mg protein (Laachari *et al*., 2013). The Km value of the *Serratia marcescens* lipase when tributyrin was used as substrate was 1.35 mM (Abdou, 2003)

3.3. pI and Molecular weight

The enzyme lipase of actinomycete NGP 1 exhibited single fraction of pI. The pI value was found to be 7.4. The molecular weight of the enzyme was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A single band was exhibited, had a molecular weight of 50 kDa. Similar to our study, the molecular weight of purified lipase enzyme was 40 kDa on SDS-PAGE from *Microbacterium sp* [9]. According to the report of Priyanka *et al*, (2019) lipase enzyme from *Pseudomonas reinekei* showed 50kDa molecular weight using negative and positive mode anion exchange chromatography.

4. CONCLUSION

The present study revealed the kinetic parameters of lipase produced from actinomycete, isolated from the South Indian coastal region. According to the kinetic studies, the enzyme will be optimized efficiently to carry out the various industrial applications.

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