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Research Article

Production, Purification and Characterization of Bacterial Lipase

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ABSTRACT

Lipase enzymes have biotechnological importance in different industrial process. The aim of this study was to purify lipase from Streptococcus sp N1 and observed the optimum conditions for enhanced production of lipase. Lipolytic activity was observed at temperature ranges 20–60 °C and pH 5–9, showing optimum activity at 30 °C at pH 8. Strain was partially purified by 60% ammonium sulphate precipitation with 654 U/ml enzyme activity. The molecular weight of enzyme was found to be 40 KDa using SDS-PAGE technique. Ca^{2+}, Fe^{2+} and Na^{2+} metal ions appeared as inducer whereas Mg^{2+} and Zn^{2+} exhibited an inhibitory effect on lipase. The enzyme was stable in EDTA, TritonX-100 and PMSF organic solvents while catalytic activity inhibited by SDS detergent. These results indicate that lipase from Streptococcus sp N1 can be a valuable candidate for industrial applications.

Keywords: *Streptococcus sp N1, Enzyme activity, Lipase, SDS-PAGE, Ammonium sulphate precipitation*

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are group of enzymes having their biotechnological potential in industries such as pharmaceuticals, food, detergent, cosmetics and textiles (Bharathi and Rajalakshmi, 2019; Martínez-Ruiz et al., 2018). Lipases act at the oil-water surface and stable in organic solvents (Atalah et al., 2019). Based on market requirement, lipases are the third largest group of enzymes after proteases and carbohydrases (Basheer et al., 2011; Martínez-Ruiz et al., 2018) Microbial lipases have attracted attention of many researchers due to desirable properties including catalytic activity at alkaline pH and high temperature, broad substrate specificity and no co-factor required for catalytic activity (Thapa et al., 2019; Bharathi & Rajalakshmi, 2019).

Over the last decade, a number of bacterial lipase have been reported from different bacterial species include *Pseudomonas* spp., *Burkholderia* sp., *Bacillus* spp., *streptococcus* sp. and *Staphylococcus* sp. (Bharathi and Rajalakshmi, 2019; Priyanka et al., 2019; Tripathi et al., 2004).

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Based on specific properties (optimal Ph, temperature and other parameters), lipases have been classified into eight families (Rios et al., 2018). Previously, Luo et al. (2006) reported psychrophilic lipases with optimal activity at 20 °C from *Pseudomonas fluorescens.*

Thermostable amylase, cellulose, xylanase and protease were produced from different bacterial strains found in Yumthang hot spring of eastern Himalaya (Sahay and Chouhan, 2018). Later on, exploration of the microbial diversity from Himalayan regions to study industrial importance reported by Goden et al. (2019). Similarly, a cold active lipase produced from *Microbacterium luteolum* isolated from Western Himalaya which showed activity at 5 °C but thermostable up to 35 °C (Joseph et al. 2012). Characteristics of lipase enzyme viz. molecular weight, activity at optimum pH and temperature, greatly depend on species and genus of microorganism (Rios et al., 2018).

The lipase producing *Streptococcus* sp. N1 was isolated from a non-thermophilic habitat (garden soil), sequenced and submitted to GenBank (ID 167506) by Tripathi et al. in 2004. The present study was aimed to purify lipase from the *Streptococcus* sp N1 and further characterized by studying lipase activity at different pH and temperature, solvent tolerability and inhibitory effect on lipase using different bivalent metal chelating agents. This study seeks to enhance the better lipase producing abilities and determine the optimal conditions for scale-up production.

MATERIAL AND METHODS

Streptococcus sp N1 strain was collected from Biochemistry division, Central Drug Research Institute (CDRI), Lucknow. Scrap from glycerol stock stored at -80°C was taken and inoculated in Luria Bertani (LB) medium. The LB tube incubated at 30 °C for 24 hrs in shaker and then culture was used for plating and further analysis. For detection of lipase production, active culture was streaked on Luria–Bertani agar plates containing 1% tributyrin (khosla et al., 2017) and on another plate Luria-Bertani agar plate without tributyrin. Streaked plates were incubated for 48hrs at 30 °C and observed plates for clear zone around the colony (Ertuğrul et al., 2007).

Lipase assay

Lipolytic activity of lipase was measured by using p-nitrophenylpalmiltate (pNPP) as substrate. The substrate solution was prepared by adding 400µl of solution A (20 mg pNPP dissolved in 20 ml of isopropanol) to 3 ml of solution B (90 ml of 50mM Sodium phosphate buffer was taken and divided into two equal parts. Dissolved 100 mg gum acacia in one part and 380µl of Triton X 100 to another part. Mixed both the solutions).The reaction mixture consisted of 20 µl of enzyme extract, 80 µl autoclaved triple distilled water and 3.4 ml of substract solution. The reaction mixture containg tubes were incubated at 30 °C for 30 min and absorbance of mixture was measure at 410 nm using spectrophotometer (Khosla et al., 2017). One enzyme unit was defined as the amount of enzyme required to release one µmole of p nitrophenol per minute from pNPP.

Protein estimation

The total protein concentration at different stages of purification was measured at 625 nm and calculated from a standard curve using Bovine Serum Albumin (BSA) following Lowry's method (Lowry et al., 1951).

Partial Purification of lipase using ammonium sulphate

Isolate was grown on Luria-Bertani medium for 24 hrs and The culture was pelleted by centrifugation at 5000 rpm for 10 min. Pellets were dissolved in 10 ml of 200 mM phosphate buffer and sonicated to get whole cell lysate. The whole cell lysate was centrifuged at 12000 rpm for 1 hr and cell free broth was separated. 60 % ammoniunm sulphate was added to cell free supernatant for protein precipitation at 4 °C with continuous string followed by centrifugation at 8000 rpm for 20 min. Semi-purified enzyme fraction collected from 60 % precipitation, lipase activity was measured by spectrophotometric enzyme activity method.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

For the detection of molecular weight of active fraction enzyme, SDS-PAGE was performed in polyacrylamide slab using 12% (W/V) resolving gel and 5% (W/V) stacking gel as mentioned by Laemmli (1970).

Effect of pH on lipase activity

The effect of pH was determined by measuring the enzyme activity with pH ranging from 5 to 10 using different pH buffer solutions (Sodium citrate for pH 5-6 and Tris- HCl for pH 7-9) at 30 °C. The enzyme activity of lipase was calculated spectrophotometrically using pNPP as substrate.

Effect of temperature on enzyme activity

Purified lipase was incubated at different temperature ranging between 20 ° C - 60 °C for 24 hrs and the relative activity of enzyme was calculated. The Reaction mixture without lipase enzyme served as control. The optimum temperature was determined which is required for maximum lipolytic activity.

Effect of enzyme activity in presence of different metal ions

Partially purified enzyme was pre-incubated in the presence of 1Mm $MgCl_2$, $MgSO_4$, $ZnSO_4$, NaCl, $FeSO_4$ and $CaCl_2$ for 1hr at optimum temperature pH values. Spectrophotometric lipase activity assay was performed for analyzing effect of metal ions.

Effect of enzyme activity in the presence of different inhibitors and detergent

Activity of partially purified lipase enzyme was analyzed in the presence of 0.4% Triton X-100, 1mM phenylmethyl sulfonylfluoride (PMSF), 0.1% SDS detergent and 1Mm EDTA. Relative activity was calculated against control using spectrometer after the 1 hr incubation period.

RESULT AND DISCUSSION

Around 90% of industrial enzymes obtain from microbial source and after carbohydrate-active enzymes and proteases, lipases are 3rd most in demand in the global industrial enzyme (David, 2017; Javed et al., 2018). In this study, *Streptococcus* sp N1 showed clear halo zone around the colony after 48 hrs incubation using LB media containing 1% tributyrin (Fig 1 & 2). Halo clear zone indicated lipase production as a result of hydrolysis of tributyrin. *Streptococcus* sp N1 lipase was purified and saturated by using ammonium sulphate precipitation method. In the process of lipase purification, it was observed precipitation occurred at 60% saturation and exhibited 654 U/ml enzyme activity.

Molecular weight of the partially purified lipase from *Streptococcus s*p N1 was found to be around 40 kDa, determined by SDS-PAGE on 12% polyacrylamide gels (Fig 3). Experimental study on pH stability of purified lipase of *streptococcus* sp N1 exhibited optimum activity pH 8.0 with 1534 U/ml. At acidic condition of pH 5 and 6, lipase activity found to be 19 U/ml and 194 U/ml respectively. Strain showed decline in lipase activity at pH 9 with 864 U/ml and further increase in pH (Fig 4). Bacterial lipase usually has an optimum pH 7 to 9 while lipase enzyme isolated from *P. gessardii* reported with an acidic optimum at pH 5.0 (Mobarak-Qamsari et al., 2011).

The optimum temperature of the purified lipase was determined by incubating enzyme at different temperatures ranges from 20 °C to 60°C by using ρ-nitrophenyl palmitate as substrate. Lipase exhibited good stability at 30 °C to 40 °C and noted optimum temperature at 30 °C with enzyme activity of 1864 U/ml. Similar to this study, *Aspergillus carneus* lipase found optimum activity at 37°C (Saxena et al., 2003) and *Aspergillus japonicus* lipase exhibite optimum activity at 40 °C (Jayaprakash & Ebenezer, 2012). Lipase with optimum activity at 50 °C from *P. helmanticensis* was reported (Phukon et al., 2020). Zhang et al in 2015 reported optimum activity of lipase at 5 °C from *Pseudomonas* sp PF 16. In present study, the enzyme activity at 20, 30 and 40 °C was observed 428, 1864 and 1424 U/ml and activity gradually reduced further at 50, 60 and 70 °C with enzyme activity activity of 628, 8 and 0 U/ml.

In present study, effect of different metal ions on lipase enzyme investigated by

incubating at 40 °C. Ca^{2+}, Fe^{2+} and Na^{2+} metal ions appeared to be the best inducer of lipase yielding with relative activity 100.5, 103 and 95% respectively (Table 1). Previously, lipases from *Pseudomonas* sp. TB11 reported positive effect of Na^{2+} metal ion (Dong et al., 2015) and *Streptomyces* sp. OC119-7 lipase were activated by Ca^{2+} (Ayaz et al., 2015). However, *S. platensis* lipase, which is inhibited by Fe^{2+} (Demir and Tükel, 2009) unlike to this study. Besides, *Streptococcus* sp N1 lipase was inhibited by Mg^{2+} and Zn^{2+} metal ions. Similarly, lipase from *Bacillus licheniformis* showed negative effect of Mg^{2+} (Jain and Mishra 2015).

The effects of various organic solvents on lipase activity were studied. Relative activity of enzyme enhanced by 103% in the presence of EDTA at 40 C°. The enzyme retained its activity by 100 and 91% in the presence of tritonX 100 and PMSF respectively (Table 2). EDTA, tritonX 100, PMSF found no inhibitory effect on *streptococcus* sp N1 lipase whereas the anionic surfactant SDS exhibited inhibitory effect on lipase with negligible relative activity. Similar to this, lipase derived from *Burkholderia ambifaria* YCJ01 activated by in the presence of EDTA and PMSF (Yao et al., 2012). While in other study, lipase purified from *Aneurinibacillus thermoaerophilus* reported inhibitory effect by EDTA and PMSF (Masomian et al., 2012).

Fig 1. *streptococcus* sp. cultivated at LB agar plate without tributyrin

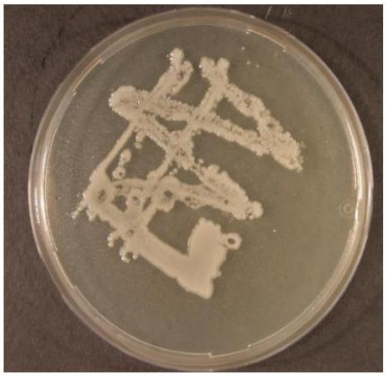


Fig 2. *streptococcus* sp. showing halo colonies with 1% tributyrin LB plate

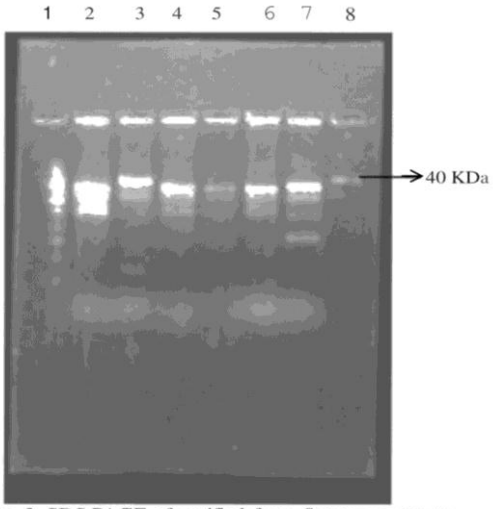
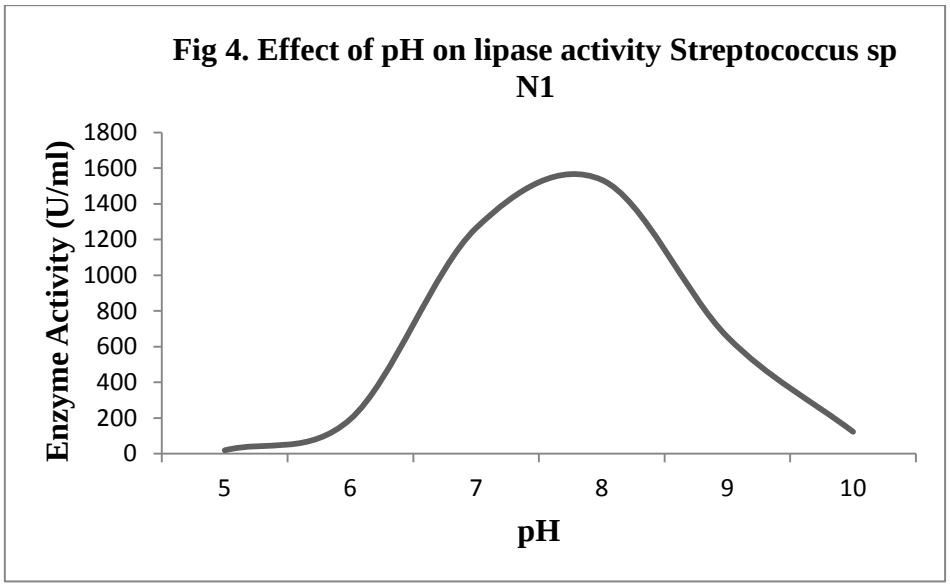


Fig 3. SDS PAGE of purified from *Streptococcus* sp
Lane 1- standard Molecular weight marker
Lane 2- crude lipase
Lane 3- cell free broth
Lane 4- whole cell lysate
Lane 5- pellets after sonication
Lane 7: sup after precipitation
Lane 8: pellet after precipitation



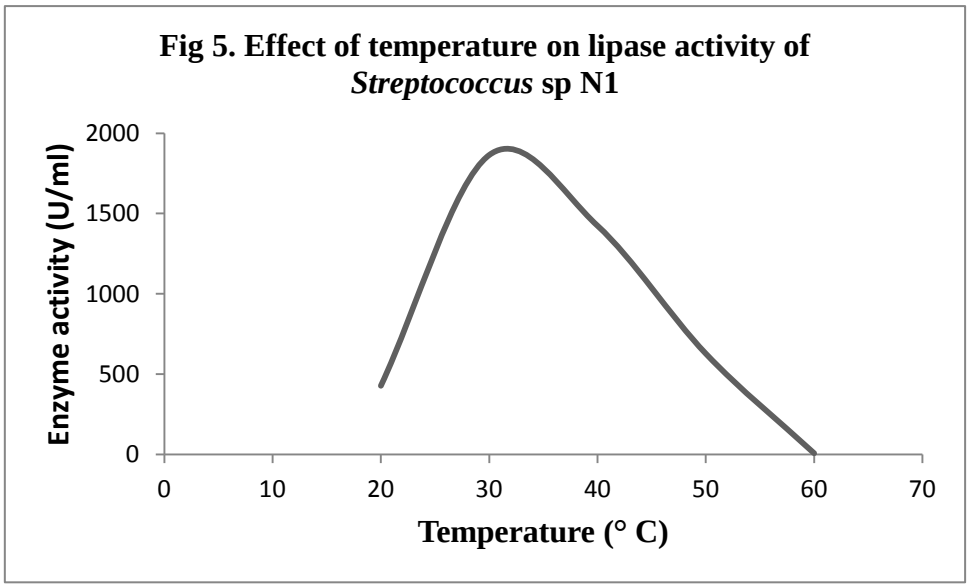


Table 1: Enzyme activity in the presence of different metal ions

S.No.	Metal ions	Final Concentration (Mm)	Relative activity (%)
1	Control	Nil	100
2	$CaCl_2$	1mM	100.5
3	NaCl	1mM	95
4	$FeSO_4$	1mM	103
5	$MgSO_4$	1mM	Negligible
6	$MgCl_2$	1mM	Negligible
7	$ZnSO_4$	1mM	Negligible

Table 2: Enzyme activity with different inhibitors and detergents

S.No.	Inhibitors	Final Concentration	Relative activity (%)
1	Control	Nil	100
2	EDTA	1Mm	103
3	Triton X-100	0.4%	100
4	PMSF	1Mm	91
5	SDS	0.1%	Negligible

CONCLUSION

In this study, it's potential for catalyzing esterification reactions at broad range of temperatures make lipase a favourable candidate for industrial application. The lipase purified from *Streptococcus* sp N1 showed activity ranging from 20 °C to 60 °C with optimal activity at 30 °C. The enzyme has molecular weight of 40 KDa determined by SDS-PAGE technique. Ca^{2+}, Fe^{2+} and Na^{2+} metal ions appeared to be the best inducer whereas Mg^{2+} and Zn^{2+} exhibited an inhibitory effect on lipase. The enzyme was stable in EDTA, TritonX-100 and PMSF organic solvents while catalytic activity inhibited by SDS detergent. *Streptococcus* sp N1 lipase with promising characteristics including broad ranges of temperature resistance, pH tolerance, active in various organic solvent and substrate specificities makes it an appealing candidate for use in detergent industries and biodiesel production industries, which require high temperatures and pH during the production processes.

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