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PURIFICATION AND CHARACTERIZATION OF EXTRACELLULAR LIPASE FROM A NEW STRAIN – *PSEUDOMONAS AERUGINOSA* SRT 9

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ABSTRACT

An extra cellular lipase was isolated and purified from the culture broth of *Pseudomonas aeruginosa* SRT 9 to apparent homogeneity using ammonium sulfate precipitation followed by chromatographic techniques on phenyl Sepharose CL- 4B and Mono Q HR 5/5 column, resulting in a purification factor of 98 fold with specific activity of 12307.8 U/mg. The molecular weight of the purified lipase was estimated by SDS-PAGE to be 29 kDa with isoelectric point of 4.5. Maximum lipase activity was observed in a wide range of temperature and pH values with optimum temperature of 55ºC and pH 6.9. The lipase preferably acted on triacylglycerols of long chain (C14-C16) fatty acids. The lipase was inhibited strongly by EDTA suggesting the enzyme might be metalloprotein. SDS and metal ions such as Hg^{2+}, Zn^{2+}, Cu^{2+}, Ag^{2+} and Fe^{2+} decreased the lipase activity remarkedly. Its marked stability and activity in organic solvents suggest that this lipase is highly suitable as a biotechnological tool with a variety of applications including organo synthetic reactions and preparation of enantiomerically pure pharmaceuticals. The Km and Vmax value of the purified enzyme for triolein hydrolysis were calculated to be 1.11 mmol/L and 0.05 mmol/L/min respectively.

Key words: *Pseudomonas aeruginosa* SRT9, extra cellular lipases, purification, Michaelis constant

INTRODUCTION

Lipases are glycerol ester hydrolases (EC 3.1.1.3), which hydrolyze ester linkages of glycerides at water-oil interface (5,34,44). An important characteristic of lipases is their ability not only to hydrolyze the ester bonds, trans-esterify triglycerides and resolve racemic mixture, but also to synthesize ester bonds in non-aqueous media (21,29). It is well known that lipases are the most widely used enzymes in organic synthesis and more than 20% biotransformations are performed with lipases (12). To date, a large number of lipases from bacteria and fungi have been extensively studied, both from the biochemical and from the genetic point of view (3,38,41). The most productive species belongs to genera *Geotrichum*, *Penicillium*, *Aspergillus* and *Rhizomucor* (30,39). Lipases from unicellular, mainly those produced by various species of genus *Pseudomonas*, have been proved to be useful both in organic reactions and in the detergent industry (17). Most of the well-studied microbial lipases are inducible extra cellular enzymes. They are synthesized within the cell and exported to its external surface or environment (40,41,45). Many of them have been purified, characterized and their encoding genes cloned (35,37,40).

Microbial lipases constitute an important group of biotechnologically valuable enzymes, mainly because of versatility of their applied properties and ease of mass production (13,28,42). Besides their industrial applications, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals and flavor compounds (16). Because of huge variation in applications, the availability of lipases with specific characteristics is still a limiting factor. Thus, to search for new lipases with different characteristics continue to be important research topics.

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In this work, we describe the production, purification and some remarkable properties of the lipase of this bacterium. Several features of the lipase regarding its substrate specificity, behavior in organic solvent, thermo stability have been reported.

MATERIALS AND METHODS

Pseudomonas aeruginosa sp. was isolated from oil and petroleum spilled soil and maintained on a growth medium containing g/L: Beef extract 1.0, Yeast extract 2.0, Peptone 5.0, NaCl 5.0, Agar 15, pH 7± 0.2 at 4ºC. The culture was periodically subcultured. For inoculum preparation the culture was reactivated by transferring into fresh broth medium containing olive oil (1% w/v) as inducer and incubated 30ºC for 24h.

Extra cellular lipase producing *Pseudomonas aeruginosa* SRT 9 (a high lipase producing strain) was identified based on cell morphology, cultural and biochemical characteristics. Further, it was confirmed by 16S rDNA technique. The sequence was edited and aligned with the sequence in the public domain Gen bank (http://www.ncbi.nih.gov) by BLAST program (4) and the organism was granted a genus and a species.

Lipase production

Production medium was prepared containing g/L: Peptone 5.0. Yeast extract 10.0, NaCl 5.0 and olive oil (1% w/v) as inducer. The initial pH of the medium was adjusted to 7.0. In Erlenmeyer flasks (500 ml) containing 100 ml of production medium, inoculum culture (1% w/v) was added and gently swirled. The inoculated flasks were incubated at 30ºC on a rotary shaker at 220 rpm for 48 h.

Lipase activity

Lipase activity was determined titrimetrically using olive oil hydrolysis (27). 1 ml enzyme solution was added to the assay substrate containing 10 ml of 10% homogenized olive oil in 10% gum acacia, 2 ml of 0.6% $CaCl_2$ solution and 5 ml of 0.2 mol/L Phosphate buffer, pH 7.0.The enzyme - substrate was incubated on orbital shaker at 150 rpm at 30ºC for 1h. 20 ml ethanol-acetone (1:1) was added to stop the reaction. Liberated fatty acids were titrated with 0.1 mol/L NaOH using phenolphthalein as indicator. The reaction mixture without the enzyme was titrated in the same way and used as blank. One 'lipase unit' was defined as the amount of the enzyme that released one µmol fatty acid per min under standard assay conditions.

Lipase activity was measured spectrophotometrically (410 nm, pH 7.0) using p-nitro phenyl palmitate (pNPP) as described by Winkler and Stuckmann (45). One unit of lipase activity was defined as the amount of enzyme that liberated 1 µmol of p-nitro phenol from pNPP per min under the assay condition.

Purification of lipase

After the incubation period, the culture was centrifuged at 15,000 rpm for 20 min at 4ºC. An enzyme preparation was obtained by precipitation with 30-90% ammonium sulphate fractionation. The precipitates were dissolved in 5 ml of Tris-HCl buffer (pH 6.8) and dialyzed overnight against 2 L of the same buffer. Each fraction was checked for enzyme activity as well as protein content. 30% ammonium sulphate fraction showing maximum lipase activity was mixed with ammonium sulphate to a final concentration of 0.25 M in 50 mM Tris-HCL buffer, pH 6.8 and applied to 15 ml of preactivated phenyl Sepharose CL-4B (1.5 X24 cm). The enzyme was eluted with linear gradient of 1% (w/v) cholate in 50 mM Tris-HCl buffer, pH 6.8 with flow rate of 1ml/min. All the fractions were checked for enzyme activity. The active fractions were pooled and applied on pre-equilibrated Mono Q HR5/5 Column (1X6cm). The enzyme was eluted by gradient NaCl (0-1.0M) in the same buffer at a flow rate of 1 ml/min. The active fractions that contained lipase enzyme were pooled, desalted and rechromatographed on Mono Q HR5/5 Column (1X6cm). The enzyme was eluted with linear gradient of NaCl (0-0.5M) in the same buffer at a flow rate of 0.5 ml/min. The lipase containing fractions were pooled and assessed for protein content. The resulting enzyme was utilized for the characterization of the extracellular lipase.

The protein content at each stage of enzyme purification was determined accordingly to Lowry et al (26) with Bovine serum albumin as the standard.

SDS-PAGE

The enzyme was electrophoresed on a 10% native polyacrylamide gel according to standard procedures (23) using a standard protein markers α-Lactalbumin (14.3kDa), Trypsin inhibitor (20.1kDa) Carbonic anhydrase (29kDa), Ovalbumin (43kDa) Bovine serum albumin (66kDa) and Phosphorylase B (97.4 kDa).

Zymogram

Zymogram study was carried according to the method proposed by Gabriel (2). Zymogram solution A contained 20 mg of α-naphthyl acetate dissolved in 5 ml of acetone to which 45 ml of 100 mM potassium phosphate buffer (pH 7) was added while stirring. Solution B contained 50 mg of Fast Red TR -salt in 50 ml of the same buffer. The solutions were prepared freshly and mixed 1:1 prior to use. The gels was incubated in the reaction mixture on a gel shaker for 5 to 15 min, on esterase activity, redness due to formation a complex between the naphthyl residue and fast red develops.

Determination of Isoelectric point

Isoelectric focusing (IEF) was carried out by IPG (immobilized pH gradient) strips with non-linear separation range of 3-10 (Amersham Bioscience) according to manufacture's instruction. Enzyme was detected using standard Commassie B staining and isoelectric point was determined according to calibrating curve supplied by the manufacturer.

Determination of Temperature and pH optima

The temperature and pH optimum of extra cellular lipase was determined at different temperatures ranging from 30-80ºC and pH values from 6 to 10.0. To determine the effect of temperature on lipase activity, purified enzyme and substrate were incubated at various reaction temperatures before starting the experiment and the enzyme assay was performed to determine the optimal temperature titrimetrically using olive oil as substrate.

The optimal pH was determined by incubating the enzyme-substrate at various pH from 3 to 10 using different buffers Sodium citrate (5-6), potassium phosphate (6-8), Tris HCL (7-9), Glycine NaOH (9-10).

Thermo stability and pH stability of lipase

Thermo stability of the enzyme was determined by incubating purified enzyme for 30 min in 50 mM Tris-HCL buffer (pH 6.8) at different temperatures (30-80ºC). The residual lipolytic activities were then determined using olive oil as substrate.

For pH stability, purified enzymes were incubated using different pH buffers. The reaction mixtures were incubated as per standard assay and the residual lipolytic activities were then determined using olive oil as substrate.

Substrate specificity

The activities of the enzyme toward various triglycerides and natural oils were investigated. Lipase activity was measured by the alkaline titration method (19). A solution was prepared by mixing 10 ml of 5% (v/v) substrate (with 1% gum arabic) 10 ml of 50 mM Tris-HCL buffer supplemented with 40 mM sodium chloride (pH 9.0), 2.0 ml of sodium deoxycholate solution (80 mg/ml pH 9.0) and 1 ml of purified enzyme in Tris-HCL buffer (pH 6.8). After adjusting pH to 9.0, the reaction was carried out at 55ºC for 1h with stirring at 150 rpm on a rotary shaker. Fatty acids liberated during incubation were titrated by 0.05 M NaOH to pH 9.0 One unit (1U) is that quantity that liberates 1 µmol of acid / min under standard assay conditions. Enzymatic activity against triolein was defined as 100%.

Substrate preference towards p-nitro phenyl fatty acyl esters was determined spectrophotometrically under standard assay conditions. The results were expressed as a percentage of the substrate that gave maximal activity.

Effect of inhibitors / activators on lipase activity

The effects of different inhibitors, salts on lipase activity were examined by measuring remaining activity after incubation with 5 mM EDTA and 2 mM concentrations of other inhibitors and salts at 30ºC for 30 min (pH 7.0) under standard assay conditions.

Effect of detergent and metal ions on lipase activity

For determining the effect of detergents and metal ions on lipase activity, the purified enzyme were preincubated with 1 mM for 30 min at 30ºC and the residual activity was determined using olive as substrate under standard assay conditions.

Stability and activity in organic solvent

The enzyme solution was mixed with different solvent solutions to yield the desired final solvent concentrations (20 & 30%). The solvents used were acetone, methanol, ethanol, iso-propanol, butanol, n-Hexane. An enzyme sample was exposed to solvents for 1 and 24h at 30ºC after which its residual enzyme activity was measured using p- NPP as substrate under standard assay conditions.

Kinetic constants

The influence of substrate concentration on the reaction velocities of the purified lipase was studied with triolein as triglyceride substrate and p-NPL and p-NPP among the p-nitro phenyl esters. The purified lipase was incubated with various concentration of emulsified triolein. The final concentration ranged from 0.5 mmol/L. For p-nitrophenylesters the final concentration ranged from 10-80 mmol/L. In all cases, the enzymatic activity was assayed under temperature and pH optima. The Michaelis constant (Km) and maximum velocity (Vmax) was determined from Lineweaver-Burk plots.

The residual activity was determined titrimetrically using olive oil as substrate using standard method. Each value in all the above experiments is the mean of triplicate experiments.

RESULTS AND DISCUSSION

Purification of Lipase

In the present work lipase produced by *Pseudomonas aeruginosa* SRT 9 in the culture broth was subjected to a purification protocol. The purification involved ammonium sulphate fractionation followed by phenyl Sepharose CL- 4B and Mono Q HR 5/5 column chromatography steps (Table 1).

The ammonium sulphate (30%) fraction was applied to FPLC phenyl Sepharose CL-4B column. Many protein peaks were observed and only one activity peak was detected (fractions 13-17). Active fractions were pooled and applied on Mono Q HR 5/5 column and the eluted enzyme showed activity peak in fraction numbers 3-4. The active fractions were again pooled, desalted and applied on the Mono Q HR 5/5 column and eluted by gradient NaCl. The fraction numbers 3-4 coincided with lipase activity peak (Fig. 1A, B). The purification process resulted in 98- fold purification factor and a final recovery (yield) of 7.53% of the enzyme with specific activity of 12307.81 U/mg.

The molecular mass was estimated to be 29 kDa (Fig. 2) with isoelectric point of 4.5 and Zymogram study revealed ester hydrolase activity. Many lipases from *Pseudomonas* sp. had a molecular mass of 29-30 kDa with pI 4.5-5.8 (7,8,36,40).

Table 1. Purification summary of lipase from *Ps. aeruginosa* SRT9.

Purification steps	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Culture filtrate	884	111024	125.59	1	100
Ammonium sulphate	5.59	14635.05	2618.07	20.84	13.18
phenyl Sepharose CL-4B	2.94	13353.02	4541.84	36.16	12.02
Mono Q column	0.68	8369.31	12307.81	98	7.53

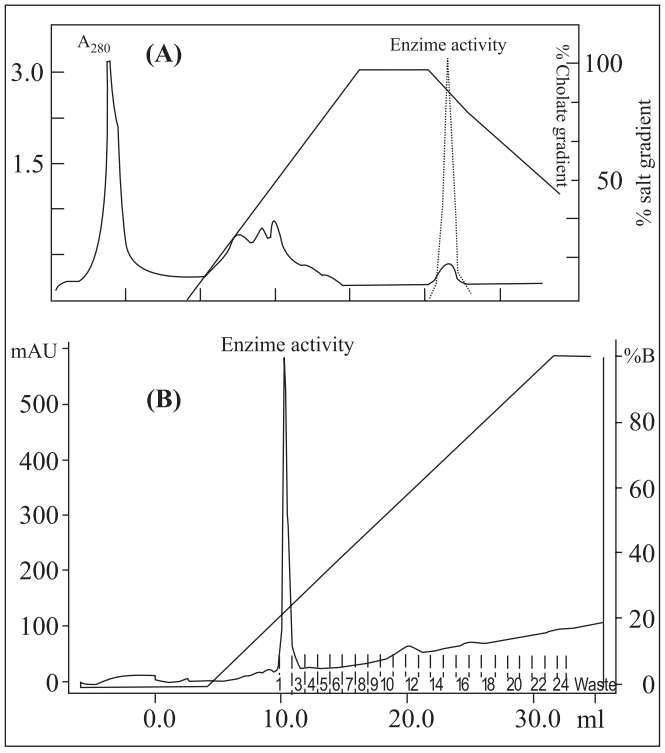

Figure 1. Elution profile of *Pseudomonas aeruginosa* SRT 9 lipase. **Figure 1A:** Chromatograph of partially purified *Ps. aeruginosa* SRT 9 lipase on FPLC with phenyl Sepharose column **Figure 1B:** FPLC profile of active fraction of *Ps. aeruginosa* lipase from Mono Q on Mono Q column.

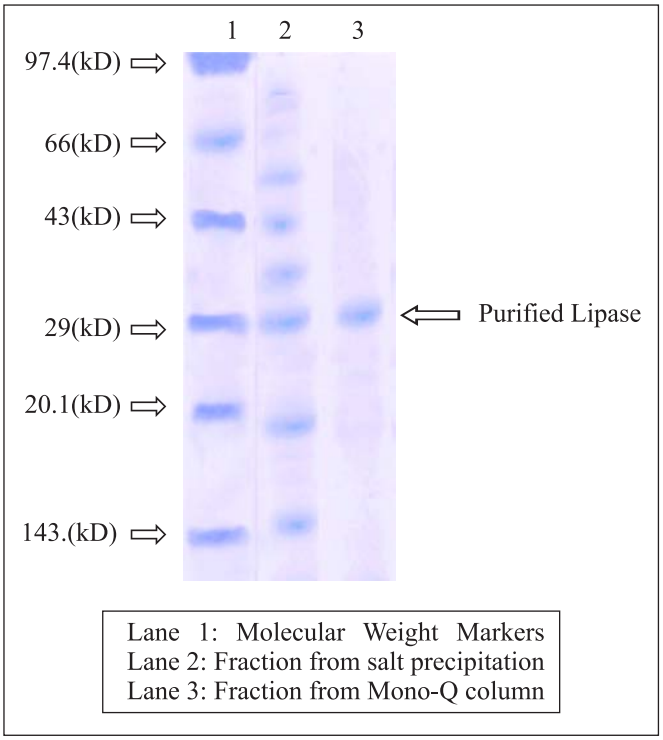

Figure 2. SDS - PAGE of *Pseudomonas aeruginosa* SRT 9 lipase purification steps.

Optimal Activity

The purified enzyme exhibited maximal activity at 55ºC temperature and pH 6.9. The enzyme was found to be fairly stable up to 65ºC, however when the upper limit was approached, the activity diminished indicating thermal denaturation (Fig. 3). The enzyme was remarkably stable in the pH range 6 -7.5 retaining more than 68% of the residual activity at pH 8 (Fig. 4). This enzyme showed to be more stable at high temperature and pH as compared to lipase obtained from *Pseudomonas aeruginosa* EF2 (50ºC and pH range 6.5-7.5) (11).

Thermal stability

The thermal stability of purified lipase was investigated at various temperatures ranging from 50-80ºC (Fig. 5). The enzyme was found to be completely stable at 55ºC after 2 h. At 65ºC, the enzyme maintained 78% of the initial activity after 1 h and 46% activity after 2h, presenting a half-life of 102 min. The half-life of this enzyme at 70ºC was 50 min. The results showed that this enzyme is more stable than lipases obtained from *Pseudomonas pseudoakaligenes* F-111 and Pseudomonas Ps-x, which showed stability upto only 70ºC (25,33).

Effect of inhibitors /activators

Lipase activity was assayed in the presence of various inhibitors and activators. The results showed that enzyme

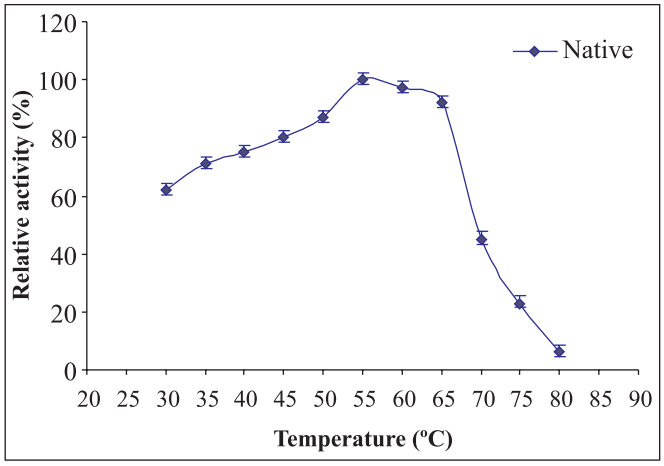

Figure 3. Effect of Temperature on Pseudomonas aeruginosa SRT 9 Lipase.

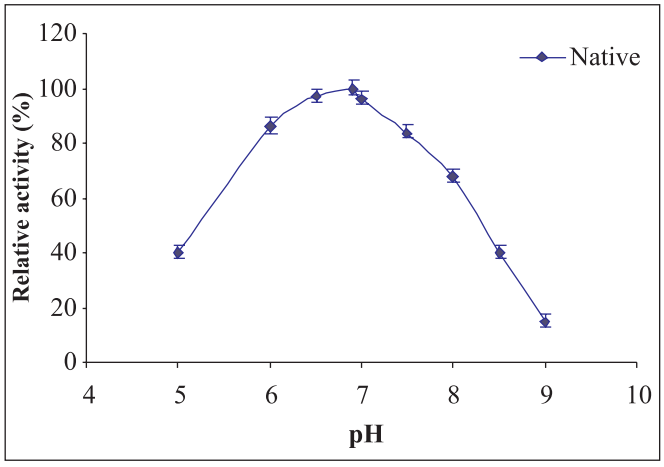

Figure 4. Effect of pH on Pseudomonas aeruginosa SRT 9 Lipase.

activity was decreased considerably in presence of 5 mM EDTA with only 36% residual activity left at 30ºC after 30 min incubation, indicating that the enzyme might be metalloprotein (9). The reducing agent, β mercaptoethanol (2 mM) and sulphydryl-reactive reagent dimethylformamide (2 mM) showed remarkable inhibitory effect with 87 and 85% residual activities respectively, indicating that the presence of - SH groups are essential for enzymatic activity (2).

Among the metal ions tested, enhancement in the enzyme activity was observed in presence of Ca^{2+} with 122% relative activity when compared to control (Table 2). Lipase enzyme have been found to be Ca^{2+} dependent and also showed increase in the thermo stability (Fig. 6). The enzyme, having lost its

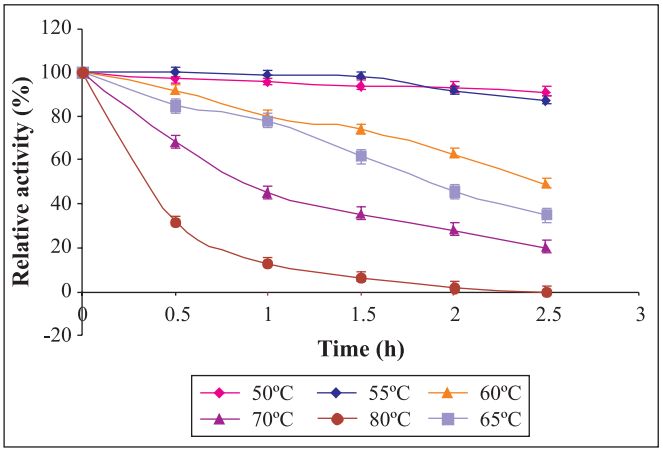

Figure 5. Effect of Temperature on lipase stability.

Table 2. Effect of different inhibitors/activators on enzyme activity.

	Reagents	Conc. used (mM)	Remaining activity (%)
Inhibitors	EDTA	5.0	36
	DMSO	2.0	89
	β-Mercaptoethanol	2.0	87
	Dimethyl formamide	2.0	85
Salts	NaCl	2.0	105
	$CaCl_2$	2.0	122
	$MgCl_2$	2.0	103

activity in presence of EDTA could be reactivated by the addition of $CaCl_2$ and there was increase in thermo stability of the lipase by about 10 degrees in comparison with lipase from *Bacillus stearothermophillus* (about 8-10 degrees) (20). Some lipases produced by *Pseudomonas* sp. have been found to be Ca^{2+} dependent, however Ca^{2+} exerted inhibitory effect on *Pseudomonas* sp. Strain S5 (32).

The effect of different detergents on the lipase activity indicated that the enzyme was fairly stable to non-ionic detergents like Tween-20, -40 and -80. Instead lipase activity was enhanced initially on their addition (~3% increase). Triton X-100 resulted in 10% decrease in the activity within 30 min. Treatment of ionic detergents like SDS resulted in remarkable loss of enzymatic activity (Table 3). SDS was also reported to be a strong inhibitor of *Bacillus thermoleovorans* CCR11 lipases and *Fusarium oxysporum* lipases causing almost total inhibition of enzyme activity (6,18). Non-ionic detergents seem to weaken the hydrophobic interaction within the protein causing disaggregation and thus stabilizing its activity while

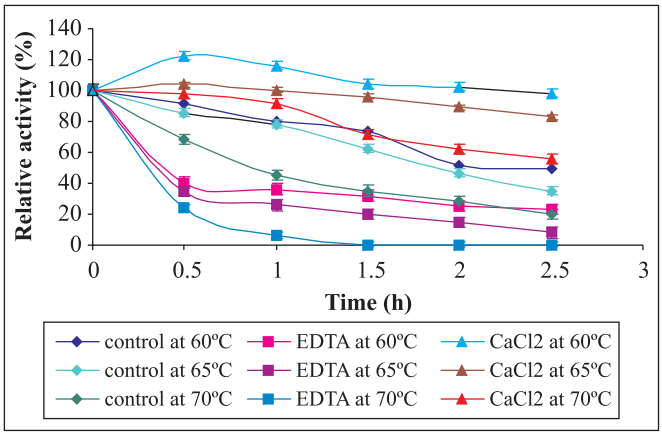

Figure 6. Effect of CaCl2 and EDTA on thermostability of lipase enzyme.

Table 3. Effect of different detergents on enzyme activity.

	Compounds	Conc. used % (w/v)	Remaining activity (%)	
			0.5h	1h
Surfactants	SDS	1.0	29.0	3.0
	Tween-20	1.0	104.5	101.0
	Tween-40	1.0	103.0	100.5
	Tween-60	1.0	90.0	84.0
	Tween-80	1.0	104.5	102.0
	Triton X-100	1.0	91.0	70.0
	Sodium-deoxycholate	10.0	99.9	97.5

SDS acts upon the di-sulphide linkages and causes inactivation/ denaturation of protein (24).

The effect of mono and divalent cations on the enzyme activity was assessed at 1 mM concentration. Ca^{2+} showed stimulatory effect whereas Mg^{2+}, Mn^{2+}, Ba^{2+} had negligible effect on the enzyme activity with ~97% relative activity. However Hg^{2+} and Pb^{2+} inhibited the enzyme activity by about 35%. Ag^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} reduced enzyme activity to less than 37%of its relative activity (Table 4). Lipases from thermophilic *Rhizopus oryzae* and *Mucor* sp. isolated from palm fruit showed very depressed activity in the presence of Hg^{2+}, Fe^{3+}, Fe^{2+} and Cu^{2+} with respect to our lipases (1,15).

Effect of organic solvents on lipase activity

Stability and activity of enzyme in organic solvents depend not only on the properties and concentration of the organic solvent, but also on the nature of the enzymes (43). Enzyme, being proteins, lose their activity after addition of organic co-solvents concentrations higher than 10-20% (14). Therefore, effect of various organic solvents at concentrations of 20% (v/v) on the enzyme was examined. The enzyme stabilities in organic solvents at 30ºC for 1 and 24 h are shown (Table 5). The results revealed that *Pseudomonas aeruginosa* SRT9 lipase was stable for 24 h in organic solvents with the exception of n-butanol and iso-propanol, suggesting alcohols with longer chain length have inhibitory effect. The lipase retains complete activity in n-hexane; even after treatment upto 48 h. A significant degree of stabilization in the presence of organic solvent has been reported for this lipase than lipases obtained from *Bacillus* (43), *Pseudomonas aeruginosa* B 11 -1 (31).

Thermo stability in organic solvents

The thermal stability of the lipase was measured as remaining activity of the purified enzyme supplemented with 30% concentration of methanol, ethanol and n-hexane at 55ºC and 70ºC (Table 6). The results revealed that enzyme retained 100% activity in n-hexane for 30 min at 55ºC and only 40% activity at 70ºC for 15 min. At 55ºC the enzyme retained 99% activity in

Table 4. Effect of different metal ions on enzyme activity.

Metal ions used	Conc. used (mM)	Remaining activity (%)
Fe^{2+}	1.0	28.5
Ag^{2+}	1.0	30.5
Cu^{2+}	1.0	32.5
Hg^{2+}	1.0	65.5
Pb^{2+}	1.0	63.5
Zn^{2+}	1.0	37.0
Ca^{2+}	1.0	107.0
Na^{+}	1.0	100.0

Table 5. Stability of lipase in different solvents.

Solvents	Conc. used (%)	Remaining activity (%)	
		1h	24h
Control	none	100	100
Methanol	20	99.5	92.5
Ethanol	20	90.5	87.5
iso-Propanol	20	60.0	50.5
Butanol	20	40.0	38.0
Acetone	20	93.5	92.0
n-Hexane	20	99. 5	99.0

Table 6. Thermo stability of lipase in organic solvents.

Organic solvent	Remaining activity (%)	
	55ºC	70ºC
Methanol	99 (for 15 min)	—
Ethanol	75 (for 15 min)	—
n-Hexane	100 (for 30 min)	40 (for 15 min)

—, Not determined.

methanol and 75% in ethanol for 15 min. However at 70ºC the enzyme was very unstable in methanol and ethanol. The thermal instability of enzymes is a consequence of protein unfolding on exposure to high temperature however an improved thermal stability of enzymes in non-aqueous media has been documented (19). Results indicate that the enzyme is clearly stable in organic solvents at 55ºC when compared to the lipases from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil (19).

Substrate specificity

The activities of the enzyme towards various triglycerides and p-nitrophenylesters were investigated (Fig. 7). With respect to the fatty acid specificity, the enzyme hydrolyzed triglycerides containing long chain fatty acids (C10-C16). Lipase showed highest activity towards triolein among the substrate examined. The enzyme also showed good activity towards trilaurin and tripalmitin with relative activities of 60%, 76% respectively. Short chain triglycerides were hydrolyzed poorly. The enzyme specificity towards lipids with fatty acid residues of C10-C16 chain length strongly suggest that the enzyme used in this study was a true lipase (18).

Kinetic constants

The Michaelis constant (Km) was determined from the Lineweaver-Burk Plot for triolein as triglyceride and p-NPL and

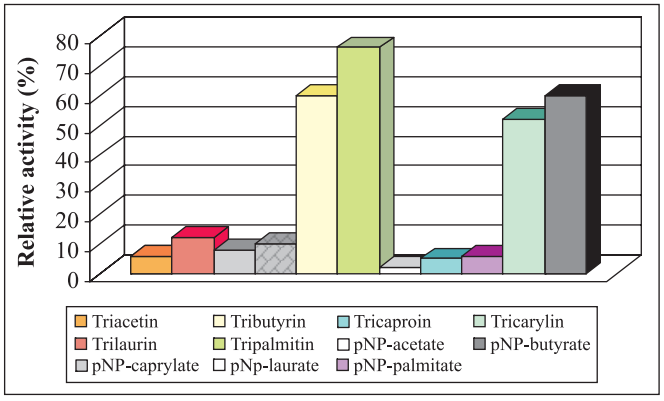

Figure 7. Hydrolytic activities of lipase with different substrates.

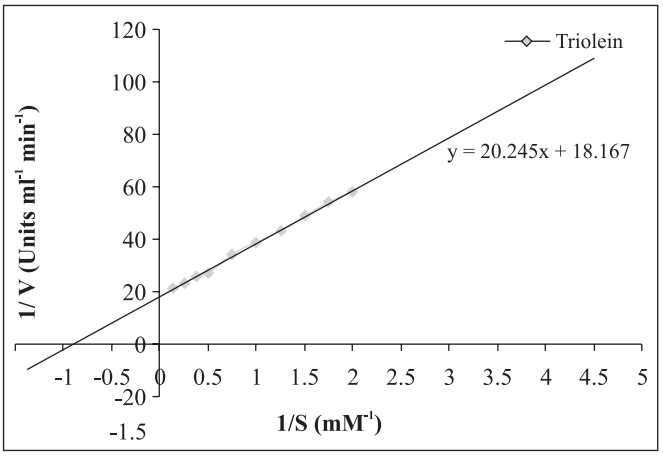

Figure 8. Lineweaver-Burk plot of Ps. aeruginosa lipases.

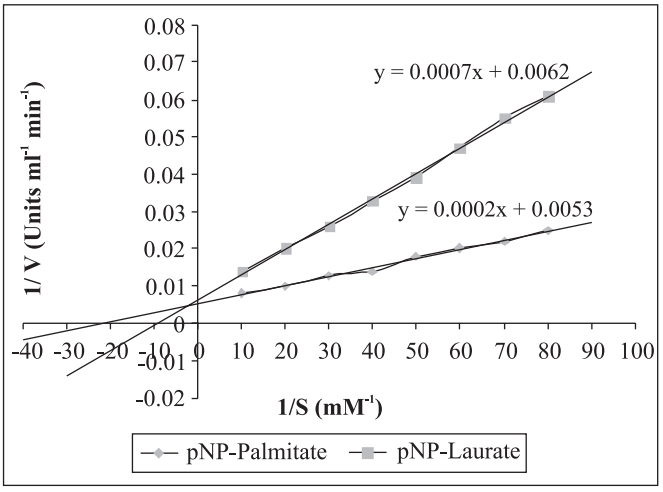

Figure 9. Lineweaver-Burk plot of Ps. aeruginosa lipase.

p-NPP as p-nitro phenyl esters (Fig. 8, 9). The Km value was determined to be 1.11 mM with triolein as substrate; Km values of p-NPL and p-NPP were 0.11 and 0.037 respectively. The Vmax was determined as the reciprocal of the intercept. The Vmax values were calculated to be 0.055 mmol/L/min for triolein and 161.3 and 188.6 mmol/L/min respectively for p-NPL and p-NPP. The lower apparent Km indicates that the partially purified lipase has higher affinity for p- NPP.

CONCLUSION

In the present work, partially purified lipase from *Pseudomonas aeruginosa* SRT 9 showed optimal activity in a wide range of temperatures and pH values. Moreover, because of its pronounced thermal stability as well as preservation of

activity and stability in organic solvents, this enzyme could be of significant biotechnological potential, particularly in organo synthetic reactions carried out at higher temperatures. Future research will focus on structural characteristics of this enzyme. Moreover, studies on sequencing, subcloning and overexpression of these genes in *E.coli* may reveal the potential applications of this enzyme in future.

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RESUMO

Purificação e caracterização de uma lipase extracelular produzida por uma nova cepa - *Pseudomonas aeruginosa* SRT9

Uma lipase extracelular foi isolada e purificada a partir de um caldo de cultura de *Pseudomonas aeruginosa* SRT9 até homogeneidade visível empregando-se precipitação com sulfato de amônia, seguida de técnicas cromatográficas em colunas de fenil sefarose CL-4B e Mono Q HR 5/5, obtendo-se um fator de purificação de 98 vezes, e atividade especifica de 12307,8 U/mg. Por SDS_PAGE, estimou-se que o peso molecular da lipase purificada é 29kDa, com um ponto isoelétrico de 4,5. A lipase apresentou atividade máxima em uma ampla faixa de temperatura e pH, com ótimos a 55ºC e pH 6,9. A lípase foi mais ativa sobre triacilglicerois de cadeia longa (C14-C16). A lipase foi fortemente inibida por EDTA, o que sugere que a enzima pode ser uma metaloproteína. SDS e íons metálicos, como Hg^{2+}, Zn^{2+}, Cu^{2+}, Ag^{2+} e Fe^{2+}, diminuíram marcadamente a atividade da lipase. Sua grande estabilidade e atividade em solventes organicos sugerem que esta lípase pode ser uma excelente ferramenta tecnológica com várias aplicações como reações organosintéticas e preparação de produtos farmacêuticos enantiomericamente puros. Os valores de Km e Vmax para a enzima purificada na hidrólise de trioleina foram 1,11 mmol/L e 0,05 mmol/L/min, respectivamente.

Palavras-chave: *Pseudomonas aeruginosa* SRT9, lipase extracelular, purificação, constante de Michaelis

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