Kinetic and Hydrolytic Characterization of Newly Isolated Alkaline Lipase from *Ganoderma lucidum* using Canola oil Cake as Substrate

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(Received on 28th August 2012, accepted in revised form 13th February 2013)

Summary: In the present work, canola oil cake was used as a substrate for lipase production employing *Ganoderma lucidum*. The enzyme was isolated and partially purified using factional precipitation, ion exchange chromatography and gel filtration. The purified lipase had a specific activity of 33262 U/mg proteins with 2.26 % recovery. The pH and temperature optima of the lipase were 8.5 and 35 °C indicating its alkaline nature. The Michaelis-Menten parameters K_m and V_{max} were found to be 0.74 mM and 4762 µmol/min respectively. Energy of activation (E_a) for PNPP hydrolysis was found to be 12.80 kJ/mol. Thermostability studies of the enzyme at various temperatures showed that the enzyme denaturation followed pseudo-first-order kinetic. Effects of various metal ions, surfactants and organic solvents were investigated. The purified lipase had the highest hydrolytic activity for waste oil indicating its potential for wide application in oleochemical and biotechnological industries.

Keywords: Lipase; *Ganoderma lucidum;* Kinetics; Thermodynamics; Hydrolysis.

Introduction

Lipases (triacyleglycerol acylhydrolase EC 3.1.1.3) comprise a group of hydrolytic enzymes which catalyze reversibly the hydrolysis and synthesis of triacylglycerides in the oil water interface [1]. Lipases are important group of biocatalysts that found a large number of applications in several industries such as food, dairy, detergent, pharmaceutical, biotechnology, oelochemical, leather etc. The most important application of lipases in oleochemical industry is the production of fatty acids from oils through hydrolysis. Free fatty acids are value added products because of their wide applications in surfactants, soap manufacturing, food industry and biomedical applications [2]. These enzymes cannot only catalyze hydrolysis reaction in aqueous media, but also can catalyze synthesis reactions such as esterification and transesterification in non-conventional media [3, 4]. Numerous important products such as flavor esters, monoacylglycerols, optically pure building blocks and also biodiesel have been produced by these reactions [5]. Lipases display little activity in aqueous solutions containing soluble substrates [6]. Today, lipases are the choice of biocatalysts as they show unique chemo-, regio, enantioselectivites, which enable the production of novel drugs, agrochemicals and fine products [7].

In recent years lipases have been subjected to active research due to their versatile applications in different industries. Mostly commercial lipases are obtained from microorganisms because microbial lipases are extracellular products and possess the ability to catalyze a wide variety of reactions in aqueous and non-aqueous phase [8]. Microbial lipases have attracted much attention because of their easy extraction, potential for an unlimited supply [9], relative ease of preparation, broad substrate specificity, including chain length selectivity, position selectivity and sterioselectivity [10]. Following proteases and carbohydrases, lipases are considered to be the third largest group based on total base volume.

Pakistan is an agro-industrial country, with large resources which can be utilized for fermentation processes. Enzymes are important products of fermentation. New sources for isolation of lipases are being carried out for a wide range of applications for their applications to withstand wide pH range and high thermal stability. There is still more scope to find new lipases with novel and specific properties. In continuous of our previous study [11] we have reported here the partial purification, kinetic and hydrolytic characteristics of a novel alkaline lipase isolated from *G. lucidum* using canola oil cake, an important waste of oil industry.

Results and Discussion

Purification of Lipases

The lipase isolated from *G. lucidum* was purified using fractional precipitation, ion exchange chromatography (DEAE cellulose column) and gel filtration. The results of lipase purification are

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summarized in Table-1. The specific activity of crude lipase was 7097 U/mg proteins. The onset of lipase precipitation occurred at 35 % while complete precipitation was recorded at 75 % of ammonium sulphate at 0 °C. The concentrated protein at 75 % saturation was collected by centrifugation at 10,000 rpm at 4 °C and dialyzed extensively against distilled water to remove the salts. The specific activity increased to 23093 U/mg proteins and 3.25 fold purification was obtained. Lipase recovered after ammonium sulphate precipitation was 63 % pure. The dialyzed pallets after ammonium sulphate precipitation were loaded on DEAE-cellulose anion exchange column and gradient elution was carried out with 0.1-0.5 M NaCl (Fig. 1). The recovery of lipase was 2.65 % and purification was increased to 3.64 fold. Specific activity was increased to 25846 U/mg.

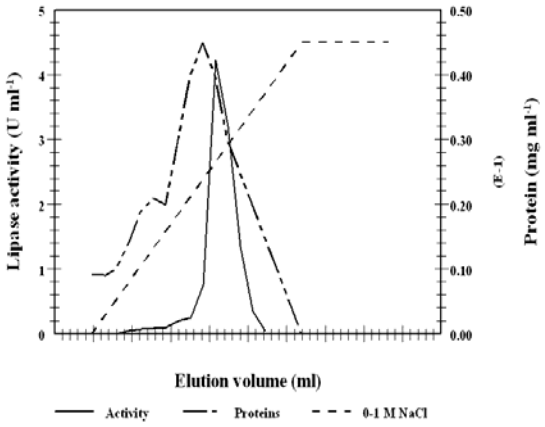

Fig. 1: DEAE-cellulose column chromatograph showing showing lipase activity and eluate volume.

The freeze dried active fractions of lipase from the ion exchange chromatography were then purified by applying the sample on Sephadex G-100 column. The enzyme purification was increased to 46.82 fold with final recovery of 2.26 %. Kashmiri *et al.* [12] purified an extracellular lipase from *Trichoderma viride* to 134-fold with an overall yield of 46 % through purification by ammonium sulphate, ion-exchange and gel permeation chromatographic techniques. Mhetras *et al.* [13] purified an extracellular lipase from *Aspergillus niger* NCIM 1207 to homogeneity level resulting in 149 fold purification and 54 % final recovery. Ramani *et al.* [14] isolated and purified an extracellular lipase from *Pseudomonas gessardii* by ammonium sulphate precipitation, DEAE cellulose column chromatography and Sephadex G-25 gel filtration. The enzyme was purified 7.59-fold with 16.2 % recovery.

Optimum pH and Temperature

The effect of pH on the activity of lipase was investigated by incubating the enzyme substrate in different buffers of pH 4-10 at 35 °C. The results indicated that the activity of lipase from *G. lucidum* increased with increase in medium pH (Fig. 2). The enzyme exhibited maximum activity at pH 8.5 which was optimum pH of lipase. However, above pH 8.5 the activity rapidly dropped. This optimum pH showed that lipase was alkaline in nature. The optimum lipase activity observed in our study is consistent with lipase isolated from the yeast *Aureobasidium pullulans* HN2.3 [15]. The optimal pH of the purified enzyme was 8.5. The effect of pH on the residual activity of lipase is also shown in Fig. 3. Lipases active and stable in alkaline media are very attracting for their great potential for applications in the detergent industry.

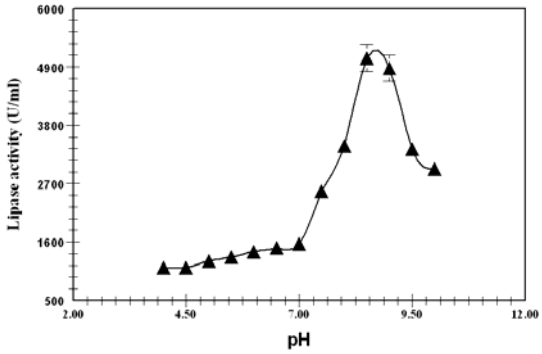

Fig. 2: Effect of pH on the activity of lipase from *G. lucidum.*

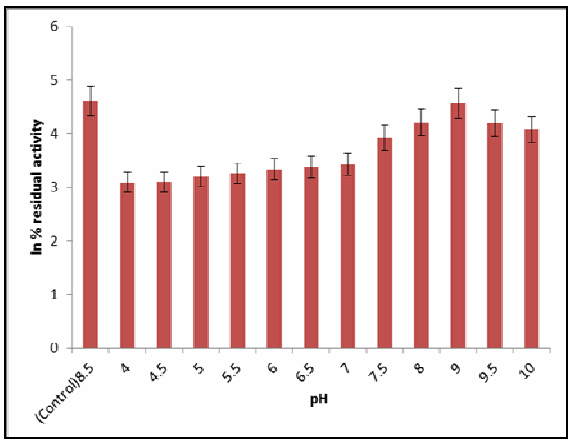

Fig. 3: Effect of pH on the residual activity of lipase from *G. lucidum.*

Table-1: Summary of the purification of lipase from *G. lucidum.*

Treatment	Total Activity (U)	Total Protein (mg)	Specific activity (U/mg)	Purification Factor	Recovery %
Crude enzyme	1575600	222	7097	1	100
$(NH_4)_2SO_4$ Precipitation	993001	43	23093	3.25	63
Ion-Exchange chromatography	40321	1.56	25846	3.64	2.55
Gel-filtration chromatography	35591	1.07	33262	46.82	2.26

Lipase activity as a function of temperature was studied in the temperature range of 25-65 °C at pH 8.5 and results are shown in Fig. 4. The results revealed that the activity of lipase increased with increase in temperature. Maximum lipase activity was recorded at 35 °C which was optimum temperature of the enzyme. A decline in the activity of lipase was observed above the optimum temperature. This decline in the activity can be attributed to the loss of catalytic activity at higher temperatures. The effect of temperature on the residual activity is also shown in Fig. 5. Hiol *et al.* [16] isolated a lipolytic strain of *Rhizopus oryzea* that yielded a high extracellular lipase activity with the temperature optima of 35 °C. Similarly Sun and Xu [17] reported that lipase from *Rhizopus chinensis* also exhibited an optimum activity in the temperature range of 35- 40 °C. The energy of activation for PNPP hydrolysis was determined by Arrhenius plot and found to be 12.80 kJ/mol (Fig. 6). Dandavate *et al.* [18] isolated an organic solvent tolerant lipase from *Burkholderia multivorans* V2 and reported that the activation energy for substrate hydrolysis was 32.5 kJ/mol,

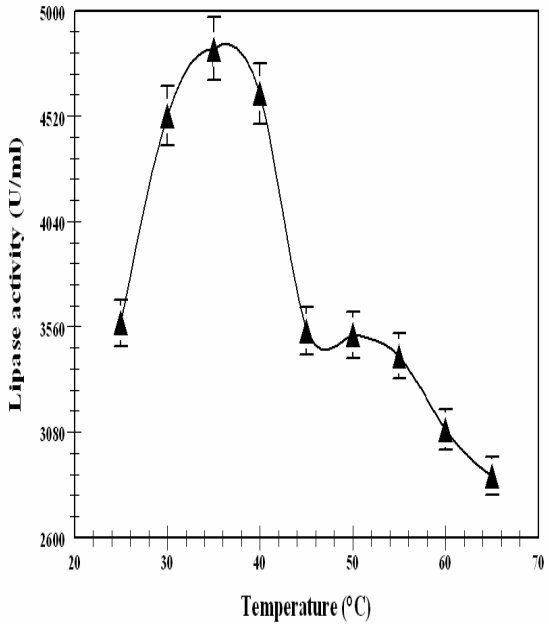

Fig. 4: Effect of temperature on the activity of lipase from *G. lucidum.*

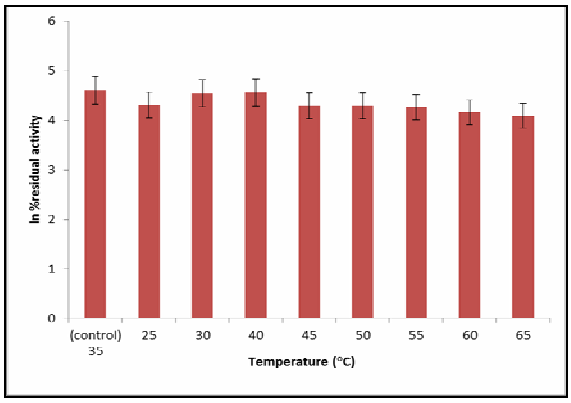

Fig. 5: Effect of temperature on the residual activity of lipase from *G. lucidum.*

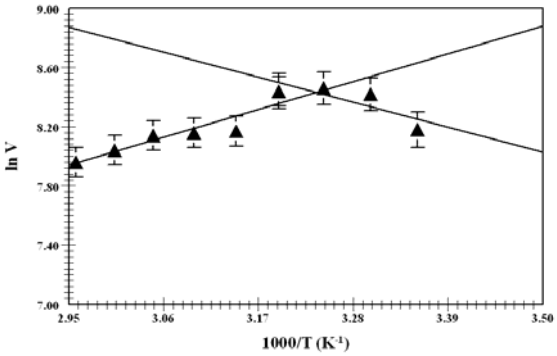

Fig. 6: Arrhenius plot for determination of activation energy for substrate hydrolysis by *G. lucidum* lipase.

Determination of Kinetic Parameters (K_m, V_{max})

Michaelis-Menten kinetic parameters, K_m, and V_{max} for the purified alkaline lipase were calculated from the Michaelis-Menten plot (Fig. 7) using different concentrations of PNPP. The K_m and V_{max} values for the alkaline lipase isolated from *G. lucidum* were found to be 0.74 mM and 4761.90 µmol min^{-1}. The lower value of K_m represents higher affinity between lipase and PNPP while V_{max} represents the higher catalytic efficiency of the alkaline lipase. Dandavate *et al.* [18] isolated lipase from *Burkholderia multivorans* V2 (BMV2). The K_m and V_{max} values for partially purified BMV2 lipase were found to be 1.56 mM and 5.62 µmoles/mg min. Kashmiri *et al* [12] cultivated an isolated a strain of

Trichderma viride for lipase production in shake flasks at 30±1 °C. The K_m value of purified lipase for triolein hydrolysis was found to be 1.229 m.mol/l.

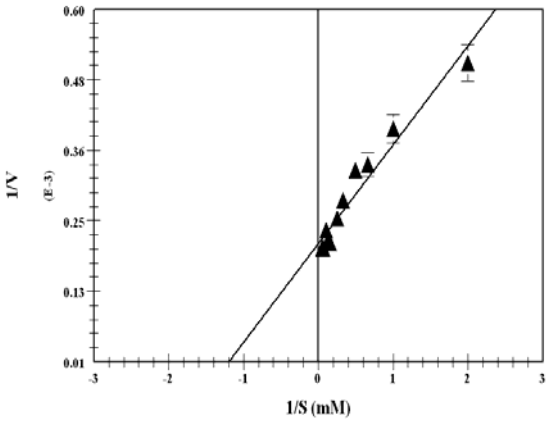

Fig. 7: Double-reciprocal plot for determination of kinetic constants of *G. lucidum* lipase.

Thermostability Studies

The rate of a reaction approximately doubles for each 10 °C rise in temperature. Assuming the enzyme is stable at elevated temperatures, the productivity of the reaction can be enhanced greatly by operating at relatively high temperatures. Consequently, thermal stability is a desirable characteristic of microbial lipases. Thermostability represents the capability of enzyme molecules to resist against thermal unfolding in the absence of substrate, while thermophilicity is the ability of enzyme to work at elevated temperatures in the presence of substrate [19]. Thermal denaturation of enzymes occurs in two steps as shown below:

$$N \leftrightarrow U \rightarrow I$$

where N is native enzyme, U is the unfolded inactive enzyme which could be reversibly refolded upon cooling and I is the inactivated enzyme formed after prolong exposure to heat and therefore cannot be recovered upon cooling. Thermal denaturation of enzyme is accompanied by the disruption of non-covalent linkages, including hydrophobic interactions, with concomitant increase in the enthalpy of activation. The opening up of enzyme structure is accompanied by an increase in disorder or entropy of activation [20]. The thermodynamic parameters for thermostability were calculated by rearranging the Eyring absolute rate equation derived from transition state theory as described by Bhatti *et al.* [21] and Amin *et al.* [22].

The results regarding the thermal denaturation of *G. lucidum* lipase studied at various temperatures (50-70 °C) are summarized in Table-2. The kinetic and thermodynamic parameters like half-life ($t_{1/2}$), denaturation constant (K_d), enthalpy of denaturation (ΔH**),** free energy of denaturation (ΔG*) and entropy of denaturation (ΔS*) were calculated from Fig. 8. The half-life of alkaline lipase was 249.3 min at 50°C and 50.22 min at 70°C. The values of enthalpy of denaturation (ΔH**)** and free energy of denaturation (ΔG**)** were 76.18 and 106.13 kJ/mol while that of entropy of denaturation (ΔS**)** was -0.0927 $Jmol^{-1}K^{-1}$ at 50 °C. The results showed a decrease in half-life and increase in denaturation constant was observed with an increase in temperature. At 70 °C, the change in free energy was 108.28 kJ/mol indicating that alkaline lipase exhibited good resistance against thermal unfolding. Thermodynamically a protein molecule is considered as more stable with high ΔG* [22]. The entropy of denaturation (ΔS*) did not show a significant change in its value indicating that there was negligible change in the configuration of alkaline lipase during unfolding at higher temperatures. Thus the *G. lucidum* lipase was thermally stable at higher temperature and could be used for industrial applications.

Table-2: Kinetic and thermodynamic parameters for irreversible thermal denaturation of lipase from *G. lucidum.*

Temp. (K)	K_d (min^{-1})	$t_{½}$ (min)	ΔH^* ($kJmol^{-1}$)	ΔG^* (kJ/mol)	ΔS^* ($Jmol^{-1}K^{-1}$)
323	**0.0028**	**249.3**	**76.18**	**106.13**	**-0.0927**
328	**0.0035**	**198**	**76.09**	**107.20**	**-0.0948**
333	**0.0077**	**90**	**76.05**	**106.67**	**-0.0919**
338	**0.011**	**63**	**76.01**	**107.32**	**-0.0926**
343	**0.0138**	**50.22**	**75.96**	**108.28**	**-0.0942**

E_a = 78.81 kJ/mol (for thermal denaturation calculated from Fig. 8
K_d = first order rate constant for thermal denaturation
$T_{1/2}$(half-life)= $0.963/K_d$

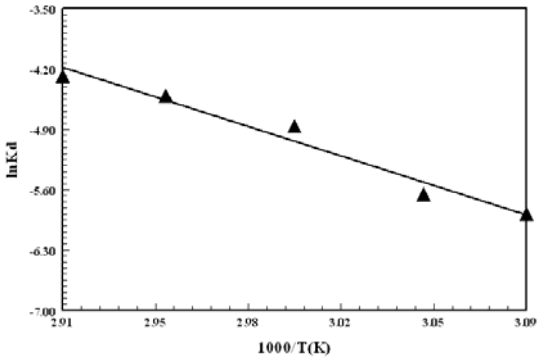

Fig. 8: Arrhenius plot for determination of activation energy for thermal denaturation of *G. lucidum* lipase.

Effect of Metal Ions, Detergents and Organic Solvents

The effect of different metals on purified lipase was examined by adding each metal ion solution (1mM) to the reaction mixture and assayed for its activity. The results regarding the effect of metal ions on the activity of *G. lucidum* lipase are shown in Table-3. It is obvious from the table that Mg^{2+} ions significantly enhanced the activity of lipase. The ions like Ca^{2+}, $Ni^{2+,}$ Mn^{2+} Cu^{2+} and Cd^{2+} showed slightly stimulatory effect. The activity of the lipase was inhibited by $Co^{2+,}$ Pb^{2+} and Fe^{3+} ions. The increase in activity of lipase due to certain ions can be attributed to the vital role played by a particular metal ion by building stable enzyme geometry as a result of metal ion binding to the interior of enzyme [14].

Table-3: Effect of metal ions on *G. lucidum* lipase activity.

Metals	Conc. (mM)	Relative activity (%)
Control (H_2O)	1	100
Cd^{2+}	1	106±1.39
Co^{2+}	1	85±14.74
Pb^{2+}	1	92±35.13
Fe^{3+}	1	91±0.069
Cu^{2+}	1	109±12.45
Mn^{2+}	1	113±4.31
Ni^{2+}	1	114±0.48
Mg^{2+}	1	122±3.61
Ca^{2+}	1	111+1.25

Rashid *et al.* [23] examined the effects of various metal ions and found that maximum lipase activity was found in the presence of Ca^{2+}. Enzyme activity was slightly activated by Sr^{2+} and inhibited by Co^{2+}, Cu^{2+} and Zn^{2+}. Hiol *et al.* [16] reported that the Na^{+}, Mg^{2+}, Co^{2+} and Mn^{2+} lightly enhanced lipase activity but was inhibited by the Fe^{2+} Cu^{2+} and Ba^{2+}.

The most commercially important field of application for hydrolytic lipase is their addition to detergents, which are used mainly in household and industrial laundry and in household dishwashers. In order to see the effect of various surfactants on the activity of alkaline lipase, the enzyme was incubated with different surfactants (1 mg/ml) and residual activity was monitored. The results showed that all the surfactants (anionic, non-ionic & commercial) slightly increased the activity of the lipase except Surf excel which inhibited its activity (Table-4). The enzyme showed good stability in various surface active agents and found that enzyme had a good stability and conformation in Tween-80, SDS and some commercial detergents. Horchani *et al.* [24] reported that lipolytic activity of the enzyme was strongly amplified in the presence of 4 mM Triton X-100.

Table-4: Effect of surfactants on lipase activity.

Surfactants	Concentration (mg/ml)	Residual activity (%)
Control (H_2O)	-	100
Tween-80	1	103.99±5.59
Triton X-100	1	99.92±5.45
SDS	1	101.28±26.30
Ariel (commercial)	1	103.12±22.74
Surf excel (commercial)	1	94.68±12.93
Lemon max (commercial)	1	106.61±6.97

The stability of lipase in organic solvents offers advantages for enzyme precipitation and synthetic applications. It is a well-known fact that organic solvents do have some stimulatory/inhibitory effects on the activity of different lipases. It depends on the source of lipases, extraction protocol and purification techniques. The relative activity of *G. lucidum* lipase studied in the presence of different polar and non-polar solvent is given in Table-5. The data indicated that the enzyme had a good stability in organic solvents like ether, benzene and spirit. It is reported that the polar solvents are not well preferred than the non-polar solvents as they cleave the thin layer of water molecules around the catalytic site of the enzymes. Our findings are in close agreement with those of Ramani *et al.* [14] in which lipase isolated from *Pseudomonas gessardii* showed good activity in non-polar solvents

Table-5: Effect of organic solvents on lipase activity.

Organic solvents	Relative activity (%)
Control (Tris-HCl buffer)	100
Acetone	27.32±2.70
Ether	104.51±4.27
Petroleum ether	8.71±0.51
Ethanol	65.41±2.69
Propanol	74.81±2.43
Hexane	12.01±0.43
Benzene	126.31±4.17
Spirit	102.05+2.99

Hydrolysis of Edible Oils

It has been reported that the hydrolytic activity is the basic characteristic of lipases. However, the same lipase has different hydrolytic activity towards different kinds of lipids from different sources. It is required that lipase should be non-specific with high specific activity, hydrolyzing different kinds of lipids from different sources when it is applied to lipase biosensor, detergent industry and digestion of lipids in the food and medicine [15]. Therefore, the purified lipase hydrolytic activity towards olive oil, sunflower oil, mustard oil and waste oil was analyzed and results are given in Table-6. The results indicated that the purified lipase from the supernatant of the fungal culture used in this

study had the highest hydrolytic activity towards waste oil although it could hydrolyze all the oils tested in this study, suggesting that lipase has high potential application in digestion of fats and lipids.

Table-6: Oil hydrolysis by the purified lipase.

Oil	Specific hydrolytic activity (U/mg protein)
Olive oil	207.51±16.30
Sunflower oil	184.46±6.7
Mustard oil	224.81±8.16
Waste oil	1106.79±81.51

Experimental

Chemicals and Substrate

All the chemicals used in the present study were of analytical grade and mainly purchased from Sigma Chemical Co. (Sigma- Aldrich, St. Louis, MO, USA), unless otherwise mentioned. Canola oil cake was obtained from a local oil Mill.

Microorganism and Lipase Production

G. lucidum employed in the current study for lipase production was obtained from the Institute of Horticultural Sciences, University of Agriculture, Faisalabad. It was maintained on potato dextrose agar (PDA) slants at 4°C. The development of inoculum was carried out by transferring spores from 4 to 5 days old slants to 500 ml Erlenmeyer flask containing 150 ml of sterile Vogel's medium with the help of a sterile loop in aseptic conditions [21]. The pH of the medium was adjusted to 5.0 using 0.1 N hydrochloric acid or sodium hydroxide solutions. The inoculum flasks were incubated at 30°C (150 rpm) for 72 to 96 h to get homogenous spores suspension (10^7 – 10^8 spores /ml). Lipase production was carried out using canola oil cake as substrate under solid state fermentation conditions as described earlier [11].

Lipase Activity Assay

The lipase activity in the culture supernatant was determined using *p*-nitrophenyl palmitate (PNPP) as substrate. Appropriate diluted enzyme (100 µl) was mixed with 0.9 ml (900 µl) of the solution containing: 3 mg PNPP dissolved in 1 ml of propane-2-ol diluted in 9 ml of the 50 mM Tris/HCl (pH=8) buffer containing 40 mg Triton ×-100 and 10 mg of gum Arabic. The stated mixture was incubated at 37°C for 30 min. The liberated *p*-nitrophenol was immediately recorded at 410 nm [25]. One unit of lipase activity was defined as the amount of enzyme required to produce 1 µmol of the *p*-nitrophenol per ml per minute under the stated assay conditions.

Protein Estimation

Total protein was estimated by using Bradford method [26]. Bovine serum albumin (BSA) was used as standard.

Purification of Lipase

Purification of lipase, isolated from *G. lucidum* was carried out by subjecting the crude enzyme solution to ammonium sulphate precipitation, DEAE-cellulose column and gel filtration chromatography. Crude lipase broth was concentrated with solid ammonium sulphate to get 75 % saturation. The precipitated protein was collected by centrifugation at 10,000 rpm at 4 ºC for 15 minutes. The pallets containing lipase was dissolved in minimum amount of phosphate buffer (pH 7) and dialyzed extensively against distilled water to remove the salts.

A column of DEAE-(Diethyl amino ethyl) cellulose (2.4 x 26 cm) was prepared, properly washed and equilibrated using with Tris/HCl buffer pH 9.5. The column was loaded with 2 ml desalted enzyme sample. The elution of the sample was carried out with a linear gradient of NaCl (0.1–0.5 M) prepared in Tris/HCl buffer pH 9.5. The drop rate of eluted sample was kept constant and 50 fractions of 2 ml each were collected. All these fractions were subjected to enzyme assay and protein estimation. The fractions having the maximum activity were pooled and dialyzed extensively against distilled water. The dialyzed fractions were freeze dried and dissolved in minimum amount of distilled water. This concentrated fraction was chromatographed on a column of Sephadex G-100 equilibrated with phosphate buffer pH 7. Elution was carried out by phosphate buffer (pH 7) at a constant drop rate of 2.5 ml/min. A total of 25 fractions were collected which were then subjected to enzyme assay and protein estimation. Samples having maximum activity were pooled together and dialyzed against distilled water. This dialyzed sample was then freeze dried and stored at -4° C till further use [22].

Optimum pH and Temperature

The effect of pH on the purified lipase was determined by incubating the enzyme-substrate at various pH ranging from 4.0-10.0 using the standard assay conditions. The buffers used were 0.1 M acetate buffer (pH 4.0 -5.5), 0.1 M phosphate buffer (pH 6.0-8.0) and 0.1 M Tris/HCl buffer (pH 8.0-10.0). The optimum temperature for the purified lipase was determined by incubating the enzyme substrate at various temperatures ranging from 25 to

65 °C under optimized pH. The activity of lipase was determined as described above.

Determination of Kinetic Parameters

The Michaelis-Menten kinetic constants such as maximum reaction rate (V_{max}) and Michaelis-Menten constant (K_m) of the purified lipase were determined by using the different concentrations of PNPP (0.5 -20 mM) under optimum pH (8.5) and temperature (35 °C). Lipase activity with each PNPP concentration was determined keeping enzyme concentration constant. The kinetic parameters were determined from Lineweaver-Burk plot.

Thermostability Studies

Thermal stability of lipase isolated from *G. lucidum* was studied by incubating the enzyme in 50 mM Tris/HCl buffer (pH 8.5) at different temperatures (50-70 °C) in the absence of PNPP as substrate. Aliquots were withdrawn at periodic intervals and cooled in an ice bath prior to assay. From a semi-logarithmic plot of residual activity vs. time, the inactivation rate constants (k_d) were calculated and apparent half-lives were estimated. The temperature dependence of k_d was analyzed from the Arrhenius plot; the activation energy for thermal inactivation (E_a) was obtained from the slope of the plot as described earlier [21, 22]. Activation enthalpy for thermal inactivation (ΔH*) was calculated according to the following equation:

$$\Delta H^* = Ea - RT \quad (1)$$

where R = 8.314 JK^{-1} mol^{-1} is the universal gas constant and T the absolute temperature. The values for free energy of inactivation (ΔG*) at different temperatures were obtained from the following equation:

$$\Delta G^* = -RTln(k_d h/kT) \quad (2)$$

where h is the Planck's constant and k the Boltzmann's constant.

Activation entropy (ΔS*) was calculated using the following equation:

$$\Delta S^* = (\Delta H^* - \Delta G^*)T \quad (3)$$

All the experiments were conducted in triplicate and results are expressed as mean ± SD.

Effect of Metal Ions, Detergents and Organic Solvents

The effect of metal ions on the activity of lipase was studied by incubating the enzyme with 1 mM solution of Cd^{2+}, Co^{2+}, Pb^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, and Mg^{2+} ions in 100 mM Tris/HCl buffer (pH 8.5) at 35 °C for 1 h. The stimulating or inhibitory effect was monitored by measuring the residual activity of the enzyme. Different detergents such as 0.1 % of SDS, Triton X-100, Tween 80 as well as commercially available detergents (Ariel, Surf excel and Lemon max) were incubated with the buffered lipase (pH 8.5) for 1 h at 35 °C. The residual activity of the enzyme was measured as described before. Similarly to see the effect of different organic solvents on lipase activity, the enzyme was incubated with 10 % solution of acetone, ether, petroleum ether, ethanol, propanol, hexane, benzene and spirit at 35 °C for 1 h and its residual activity was measured.

Hydrolysis of Edible Oils

The effect of lipase from *G. lucidum* on the hydrolysis of edible oils was investigated by incubating the enzyme with oil/fat as described by Liu *et al.* [15]. 5 mL of 0.1 M Tris/HCl (pH 8.5) and 4.0 mL of 50 % oil emulsion (2.0 mL of 2.0 % polyvinyl alcohol was mixed with the same volume of oils i.e. olive oil, mustard oil, sunflower oil and waste oil) were added to 100 mL flasks and the mixture was kept at 35±1 ºC for 5 min. 1 mL of the purified lipase was added to the mixture in each of the flasks. Another flask without addition of the purified lipase was used as the blank. The flasks were shaken at 35±1 ºC in the water bath for 30 min. 10 mL of 95 % ethanol solution was added to the mixture immediately to cease the reaction. At the same time, 1 mL of the purified lipase was added to the blank. Liberated free fatty acids were titrated with 0.05 M NaOH using phenolphthalein as indicator. One unit of hydrolytic activity of the lipase was defined as the amount of enzyme which catalyzes the release of 1µmol of free fatty acids per min under the above conditions.

Conclusion

The main purpose of the present investigation was to isolate and characterize the lipase from *G. lucidum* (white rot fungus) for its potential applications in various industries. An alkaline lipase was isolated from the fungus using canola oil cake as substrate. The optimum pH and temperature of the purified lipase was found to be 8.5 and 35 °C respectively. The ions like Mg^{2+}, Ca^{2+}, Ni^{2+}, Mn^{2+} Cu^{2+} and Cd^{2+} showed slightly stimulatory effect on the lipase activity. The anionic, non-ionic & commercial slightly increased the activity of the lipase. The highest hydrolytic activity towards waste oil although it could hydrolyze all the oils tested in this study, suggested that *G. lucidum* lipase has high potential application in digestion of fats and lipids.

Acknowledgments

The authors are thankful to the Higher Education Commission of Pakistan for financial support under Indigenous PhD Fellowship 5000 to meet this achievement.

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