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Partial Purification and Characterization of Extracellular Lipoprotein Lipase (ELPL) Obtained from *Lysinibacillus fusiformis L-52* Bacteria Isolated from Dairy Industry Wastes

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A B S T R A C T

Among various industrial enzymes, lipases have an important role in a wide variety of industrial applications. There are very few reports, that lipase was obtained from *Lysinibacillus fusiformis* L-52 bacteria isolated from dairy industry effluents. This Extracellular Lipoprotein Lipase (ELPL) released from the isolated bacteria was purified partially by using ammonium sulfate precipitation and Sephadex G-150 column chromatography. The purified enzyme was found to be a lipid-containing protein with a molecular weight of 69.89kDa approximately. It has shown the lipolytic activity on various substrates, specifically on natural substrates like olive oil and sunflower oil, and on synthetic substrate 4-para Nitro Phenyl Palmitate (pNPP). ELPL showed a Km value of 0.045mM with pNPP as substrate and has an optimum temperature at 370C, and optimum pH of 7.0. The enzyme was found to be active in hydrolyzing dairy effluent and also serum lipids. ELPL also showed a significant effect in hydrolyzing plant-based lipids containing monounsaturated fatty acids like oleic acid and linoleic acid but not on animal fats like butter.

Introduction

In the present situation, the effluents released from the industries cause a major problem to the environment. Even though dairy industries produce many economically useful products, the effluents released from these industries causes potential damage to the environment, thus it is very essential to recycle the effluents. Recycling leads to the development of products that are economically beneficial to society (Agobo *et al.*, 2017). Among those economically important products, enzymes like lipases are most important due to their wide industrial applications in various industries like pharmaceutical, cosmetics, detergent, etc. Lipases are a special group of enzymes that acts between the

hydrophobic phase and the aqueous phase. With respect to their wide range of industrial applications, the mechanism of enzyme action diverges in synthesis, inter-esterification reactions, and ester hydrolysis (Jaganmai and Rajeswari, 2020). The purification of enzymes from industrial effluents particularly involves the isolation of microorganisms specific to the production of the required enzyme.

A few bacteria are involved in producing specific enzymes to detoxify the environment by degrading the harmful complex industrial pollutants into simple products by a process known as bioremediation.

The mechanism of bioremediation involves chemical reactions like oxidation, reduction, elimination etc. For several enzymes like lipases, proteases, dehydrogenases, hydrolases, laccases etc were obtained from different microorganisms like *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus*, and *Mycobacterium* etc. (Jaganmai and Rajeswari, 2020).

Hence the isolation of useful microorganisms involved in reduction of pollutants like oils/lipids can be isolated from the effluents. But the process of microbial isolation from the effluents is a challenging task and further, the process of partial purification is involved many steps.

Hence this study mainly deals with partial purification of lipase by aqueous extraction through conventional gel filtration chromatography with Sephadex G-150 followed by the characterization to determine the optimum pH, temperature, effect of carbon source, nitrogen source, pH, temperature, metal ion concentration, and also the effect of various detergents, and reducing agents.

Materials and Methods

Instruments

Rotatoryshaker (RIA), Incubator (Remi), Laminar airflow chamber (Remi), Spectrophotometer (Infra digi), Weighing balance, pH meter, Light Microscope, PCR machine, Cooling refrigerator, Autoclave (Remi), Water bath, Hot air oven (Remi), Water Distillation unit. SDS gel apparatus (BIO RAD), UV transilluminator

Chemicals

Sodium hydroxide (KOH),Ammonium Dihydrogen phosphate (NH4H2PO4), Dipotassium Hydrogen phosphate (K2HPO4), Sodium citrate, Magnesium sulfate (MgSO4), Disodium hydrogen phosphate (Na_2HPO4), Potassium dihydrogen phosphate (KH_2PO4), Calcium chloride ($Cacl_2.H2O$), Peptone, Potassium Dihydrogen Phosphate (KH_2PO4), Disodium Potassium hydrogen phosphate ($Na2H_2PO4$), Ammonium sulfate ($(NH_4)_2SO_4$), Yeast extract, Agar, Polyvinyl alcohol (PVA), Vanillin reagent, Conc. Phosphoric acid, Cholesterol, acetone, Tween-20, Triton X-100, Fructose, Galactose, Sucrose, Sodium nitrate All the above chemicals procured form Qualigens.

DNA extraction kit, Palm oil, Mustard oil, Sunflower oil, Coconut oil, Olive oil, Butter, Peanut oil, Tributyrin (Himedia). Sephadex G 150, Ammonium sulphate, Cholesterol, acetone, Tween-20 and Butter, Fructose, Galactose, Sucrose, Sodium nitrate, Acrylamide-Bisacrylamide, Tris-HCl, SDS (Sodium dodecyl sulphate), APS (Ammonium persulphate), TEMED (N,N,N,N tetramethylethylene diamine), Glycerol, β merkaptoethanol, Glycine, Methanol, Acetic acid, Coomassie Brilliant Blue. 4-paraNitroPhenol Palmitate (4-pNPP) were purchased from Sigma.

Sample Collection

The total effluent waste is collected from the Vaishnavi dairy industry, Wyra, Khammam district, Telangana in a sterile container, and stored at 40C until the analysis was carried out according to the standard methods of APHA (American Public Health Association). The lipolytic bacteria is isolated from the total effluent wastes after enriching with Olive oil emulsion and confirmed further on

tributyrin agar medium. The colonies with a clear zone of hydrolysis were selected and subcultured in a tributyrin agar medium (Hiol *et al.,* 1999).

Extraction of enzyme

Lipolytic bacteria isolated from waste water is identified as *Lysinibacillus fusiformis* L-52 by using 16srRNA analysis. (Hiol *et al.,* 1999) The isolated lipolytic bacterial culture was inoculated in tributyrin broth and incubated at 370C at 120 rpm for 72 hrs on a rotatory shaker incubator. The broth was centrifuged at 10,000rpm for 10min at 40C to separate the bacteria. The supernatant obtained was further used as a source for crude extracellular lipase since it has maximum lipase activity, and is used for further purification and analysis.

Methods used in the determination of lipase activity

Titration method

One ml of the lipase enzyme (Crude /Partially purified) was added to a mixture of 4ml Olive oil emulsion along with 5ml of 0.5M Phosphate buffer, pH 7.0. The mixture was incubated for 15min at 370C, and the reaction was terminated by the addition of 2ml of acetone: ethanol mixture (1: 1). The reaction mixture was titrated against 0.1N NaOH by the addition of one or two drops of phenolphthalein as an indicator (Jaganmai and Rajeswari, 2020).

One lipase unit (U) is defined as the number of lipids required to liberate 1 mol of fatty acids/min. In the current study, lipase activity is measured in the presence of Olive oil as the substrate.

Lipase activity (U / ml) = ((A-B) x N (NaOH) x 1000) / (VE))

Whereas

A = Amount of NaOH required to neutralize the reaction mixture, B is for blank titration, 1000 = conversion to mmol to mol, VE = total volume of the reaction mixture.

Spectrophotometric method by 4- para nitro Phenol Palmitate (pNPP)

pNPP solution was prepared freshly by mixing 10ml of 0.03%pNPP in isopropanol, and 90ml of 0.1% Gum Arabic solution in 50mM Tris-HCl buffer (pH 8.0) containing 0.4ml of Triton X-100 under constant stirring conditions until the whole solution gets suspended.

To 0.9ml of pNPP solution (freshly prepared), 0.1ml of the lipase enzyme (Crude/Partially purified), and incubated at 370C for 30min. The color formation was measured at 410nm.

Activity (U/hr/mg of protein) = (Absorbance at 410nm/18.3) X Dilution factor

Partial purification of Extracellular lipase

The isolated bacteria was cultured in 1lit of tributyrin broth for 72hr at 370C where the production of lipase was found to be maximum. The crude extracellular lipase was collected by separating bacteria at 10,000 rpm at 40C for 10min. The supernatant was precipitated by adding Ammonium Sulfate at 100% saturation and left overnight at 40C under constant stirring in order to precipitate the protein present in the broth. Further, it was centrifuged at 10,000rpm for 10 min at 40C, and the pellet was suspended in 0.5M Phosphate buffer, pH 7.0. The suspension was desalted by using Centrikon tubes having 10 kDa cut off.

Desalted ammonium sulfate fraction was further subjected to Sephadex G-150 column chromatography which was already equilibrated with 0.5M phosphate buffer, pH 7.0. The column was eluted with 0.5M Phosphate buffer pH 7.0, and 40 fractions of 5ml each were collected with a flow rate of 1ml/min. All fractions were subjected to measure protein at 280nm and enzyme activity was checked by using pNPP method.

Characterization of partially purified lipase

Physical characterization

Confirmation for the existence of lipids in partially purified lipase

Phosphovanillin method

2ml of partially purified lipase was digested by the addition of 0.2mlof Conc. Sulphuric acid in a boiling water bath for 10min and cooled to room temperature. This digested mixture is mixed 10ml of Phosphovanillin reagent (prepared by mixing 350ml of 0.04M vanillin reagent to 50ml of water under constant stirring, and to this 600ml of Conc. Phosphoric acid (85%) is added) and incubated at 370C in a water bath for 15min (Anschau *et al.*, 2017).

Cholesterol (10mg/ml in acetone) is used as the standard to estimate the lipids present in the sample by calorimetric method and absorbance was measured at 540nm.

Thin-layer chromatography (TLC)

The lipids in the partially purified enzyme were isolated by mixing thoroughly with Chloroform: Methanol (3:1) mixture. The lipid layer was collected and subjected to Thin Layer Chromatography (TLC) by loading on silica-coated TLC plates and separated in a solvent system containing Petroleum ether: Diethyl ether: Acetic acid in the ratio of 9:1:0.1. Separated lipids were detected by using iodine crystal vapors where olive oil is used as a standard lipid.

Determination of molecular weight by SDS-PAGE

30µg of protein from crude, Ammonium Sulfate precipitated protein, and protein from pooled Sephadex G-150 fractions were loaded on 10% SDS PAGE and were over run for 30min. The molecular weight was determined by comparing to the standard protein markers, loaded along with samples in the range of (10kDa-250kDa). The molecular weight was determined from a plot between log MW and relative migration values (Rf values) of standard protein markers (Rajeswari *et al.*, 2009).

Enzyme kinetics of partially purified Extracellular Lipoprotein Lipase (ELPL)

Substrate specificity of ELPL

Different emulsified substrates like Sunflower oil, Olive oil, Coconut oil, Mustard oil, Palm oil, Butter, and Tween 20 were used to determine the substrate specificity of partially purified ELPL. The activity was determined by preincubating the aliquots of partially purified ELPL in the titrimetric method.

Effect of substrate concentration of ELPL

The enzyme activity is checked by using pNPP, as a substrate at a wide range of concentrations (10µg to 100µg) by spectrophotometric method. It is also used to determine the K_m value of the substrate.

Determination of optimum pH for ELPL

Among all the buffers (like Acetate buffer, Tris buffer, and Phosphate buffer), the Phosphate buffer is found to be having stable activity. Hence the optimum pH of lipase activity is determined by using pNPP as a substrate and preincubating the aliquots of partially purified ELPL in 0.5M Phosphate buffer at different pH ranging from pH 6.0- 8.0.

Determination of optimum temperature for ELPL

The optimum temperature for ELPL is determined by measuring the activity by using pNPP as a substrate at different temperatures ranging from (250C to 1000C), and the activity was checked by preincubating the aliquots of partially purified ELPL in the spectrophotometric method.

Effect of metal ions on ELPL

The effect of different metal ions like Na+, K+, Mg+2, Ca+2, Zn+2, Fe+2, and metal chelator EDTA at 1mM concentrations, was checked by including them separately in the enzymatic reaction mixture, and by preincubating, the aliquots of partially purified ELPL then the activity was determined by using the pNPP method.

Effect of different Detergents and Reducing agents on ELPL activity

The effect of Detergents (SDS), Surfactants (Triton X-100), and Reducing agents (β-mercaptoethanol (β-ME), and Dithiothreitol (DTT)) in the range of 1-10mM were considered in the lipase assay, and the activity was measured by preincubating the aliquots of partially purified ELPL in the spectrophotometric method.

Industrial and clinical applications of partially purified ELPL

Partially purified ELPL was analyzed for the hydrolysis of lipids and effluents collected from the industrial wastes and human serum.

Effect of ELPL on Serum lipids

Phosphovanillin method

Serum lipids from 5ml of human serum were separated by mixing with Chloroform: methanol (3:1), then the lipid layer is collected, and allowed to dry. Further, the lipids were reconstituted in 0.5M Phosphate buffer and used as a substrate for ELPL activity. Total serum lipids were estimated before and after treatment with 100µl of partially purified ELPL by using the Phosphovanillin method.

Thin Layer Chromatography (TLC)

Similarly, the Serum lipids collected before, and treatment with ELPL were loaded on silica gel coated TLC plates and were scheduled for Thin Layer Chromatography (TLC), as described previously.

Effect on dairy industrial effluent

To estimate the total lipid content present in dairy industry effluents before, and after the treatment with ELPL, by the Phosphovanillin method described previously, for the effluents were collected from different dairy industries like Vaishnavi Dairy Industry, Wyra, Khammam district, Telangana; Vijaya Dairy Milk Factory, Vijayawada, A.P; Heritage Dairy Industry, Ramachandrapuram, West Godavari district, A.P; Tirumala Milk Dairy, Guntur, AP; Sangham Milk dairy, Guntur, AP.

Results and Discussion

Culturing and isolation of extracellular lipase producing bacteria

Isolated lipolytic bacteria *Lysinibacillus fusiformis* L-52 is cultured in the medium containing Olive oil emulsion as described previously (Hiol *et al.,* 1999). It was observed that the lipase is released extracellularly and is found to be 129.6 U/hr/mg of protein by titration method, whereas the activity is found to be 52.8U/hr/mg of protein by the pNPP method.

The titration with olive oil emulsion as substrate has shown more activity due to the presence of more amount of unsaturated fatty acid in its composition compared with synthetic substrate like 4- Para Nitrophenol Palmitate.

Partial purification of ELPL

ELPL is partially purified by precipitating with Ammonium Sulfate at 100% saturation, followed by Gel filtration using Sephadex G-150 column chromatography (Fig 1).

As shown in Table 1, 100% Ammonium Sulfate precipitation showed a specific activity of 43.3 U/mg with 3.2 purification folds when compared to

crude. When subjected to the Sephadex G-150 column, it was observed that only three fractions 19, 20, 21 out of 40 fractions have shown the maximum activity and the pooled fractions showed a specific activity of 57.97 U/mg of protein with 4.39 purification folds (Table 1).

Characterization of partially purified ELPL

Analysis of lipid content in the partially purified ELPL

Partially purified ELPL when analyzed for lipid content qualitatively by TLC method and subsequently quantified by Phosphovanillin method. When subjected to TLC along with standard Olive oil, lipid content in ELPL appeared as a brown spot where Olive oil is use as a standard (Fig 2).

It was also measured as 0.08µg of lipid content present in 1ml of partially purified lipase enzyme quantitatively by the Phosphovanillin method and the results are compared to that of standard Cholesterol.

Determination of molecular weight by SDS-PAGE

SDS-PAGE analysis showed the presence of a major band in all Crude, Ammonium Sulfate, and Pooled Gel filtration fractions. The molecular weight of the major band is calculated based on the -log value of standard molecular weight protein markers and Rf values based on their mobility. It was found to be 68.98 kDa as shown in the Fig 3.

Enzyme kinetics of partially purified lipase

Substrate specificity of partially purified ELPL

Partially purified ELPL activity was checked for its activity on the various natural plant, and animal lipids, where several plant oil substrates (Olive oil, Sunflower oil, Mustard oil, Coconut oil, and Palm oil) and animal oil substrates like Butter along with a detergent-like Tween 20 were used for titration method as described in materials and methods (Fig 4A). The results showed that hydrolysis activity is maximum for Sunflower, and Olive oil when compared to other substrates.

It is measured as 175±0.06 U/hr/mg and 173±0.06 U/hr/mg of protein respectively. However, the hydrolysis capacity is minimum for animal fats (butter) measured as 162±0.06 U/hr/mg and also 114±0.06 U/hr/mg for Tween 20 (detergent).

The activity is also measured with pNPP, a standard substrate for lipase activity, where it is observed to be 22.2±0.04 U/hr/mg. And further kinetic studies were measured by using pNPP, as it is a standard substrate.

Effect of substrate concentration on ELPL

When ELPL is subjected to different concentrations of pNPP, it is observed that the rate of reaction increases linearly up to 40µg of pNPP. Further, it is stabilized from 0.000108mM to 0.000269mM substrate concentration.

Based on the graph it is observed the maximum velocity was observed as 90 U/hr/mg of the enzyme at the concentration of 40µg of pNPP. When calculated for 1/2Vmax it was found to be 45 U/hr/mg (Fig 4B).

Determination of optimum pH for ELPL

The optimum pH of ELPL was determined by using the pNPP as a substrate and observed that the activity increases steadily up to pH 7.0 and further decreased due to an increase in pH of 0.5M Phosphate buffer. As shown in Fig 4C, it is observed that the activity is maximum at pH 7.0 with 26±0.03 U/hr/mg.

Determination of optimum temperature for ELPL

The optimum temperature of partially purified ELPL is checked for hydrolysis at different temperatures

and is observed to be optimum at 37^0C where the activity is found to be 25.8±0.04 U/hr/mg (Fig 4D).

The results clearly showed that the increase in activity with an increase in the temperature from 25^0C up to 37^0C, and further it was decreased rapidly due to an increase in the temperature beyond 37^0C.

Effect of metal ions on partially purified ELPL

The effect of different metal ions (Mg^{+2}, K^+, Na^+, Ca^{+2}, Zn^{+2}, Fe^{+2}) and Chelating agent (EDTA) at 1mM concentration was checked on ELPL activity.

It is observed that the activity remained more or less equal to that of control (25.2±0.06 U/hr/mg) when treated with Mg^{+2} and Na^+.

However, it is observed that the activity is inhibited with the addition of K^+, Ca^{+2}, Zn^{+2}, and Fe^{+2} as shown in Fig 5A. It is observed that the enzyme activity didn't show any effect upon treatment with EDTA. From the graph, it is clearly observed that 59%, 54%, 72%, and 72% of inhibition for K^+, Ca^{+2}, Zn^{+2}, and Fe^{+2} respectively.

Effect of different detergents and reducing agents upon partially purified ELPL

When checked for the effect of different inhibitors like Detergents (Sodium Dodecyl Sulfate (SDS)), Surfactant (Triton X-100), Thiol reducing agents (Dithiothreitol (DTT), β-Mercapto Ethanol (β-ME)) upon ELPL activity. Upon treatment with reducing agents like DTT, and β-ME, it is observed that the activity is inhibited when compared to control.

It has shown clearly that 18.9% of inhibition at 1mM concentration, and 89% of inhibition at 10mM of DTT. Similarly, with β-ME, it is observed that 70% of the activity is inhibited at 1mM concentration (Fig 5B). Further, it is also observed 64.5% and 95% of inhibition with 10mM SDS and Triton X-100 successively when compared to control.

Industrial and clinical applications of partially purified ELPL

Industrial applications

It is observed that the partially purified ELPL has shown its activity on lipid pollutants existing in dairy industrial effluents. It is clearly observed that the lipid content from various dairy industries (Vaishnavi Dairy Industry, Wyra, Khammam district, Telangana; Vijaya Dairy Milk Factory, Vijayawada, A.P; Heritage Dairy Industry, Ramachandrapuram, West Godavari district, A.P; Tirumala Milk Dairy, Guntur, AP; Sangham Milk dairy, Guntur, AP) is hydrolyzed completely to 98-99%, after the treatment with ELPL (Fig 6(b)).

From Fig 5A, it can be shown that the lipid pollutes in the dairy industrial effluents hydrolyzed completely and brought the effluent almost to the lipid-free condition.

Table.1 The table showed the steps involved in the partial purification of lipase

S. No.	Purification steps	Total protein (mg/ml)	Total activity (U/ml)	Specific activity (U/mg)	Purification Fold	Yield (%)
1	Crude extract	700	9250	13.2	1.00	100
2	Ammonium Sulfate Precipitation	12	520	43.3	3.2	5.6
3	Sephadex G-150	5.175	300	57.97	4.39	3.2

Fig.1 Chromatogram of column chromatography by using Sephadex G-150

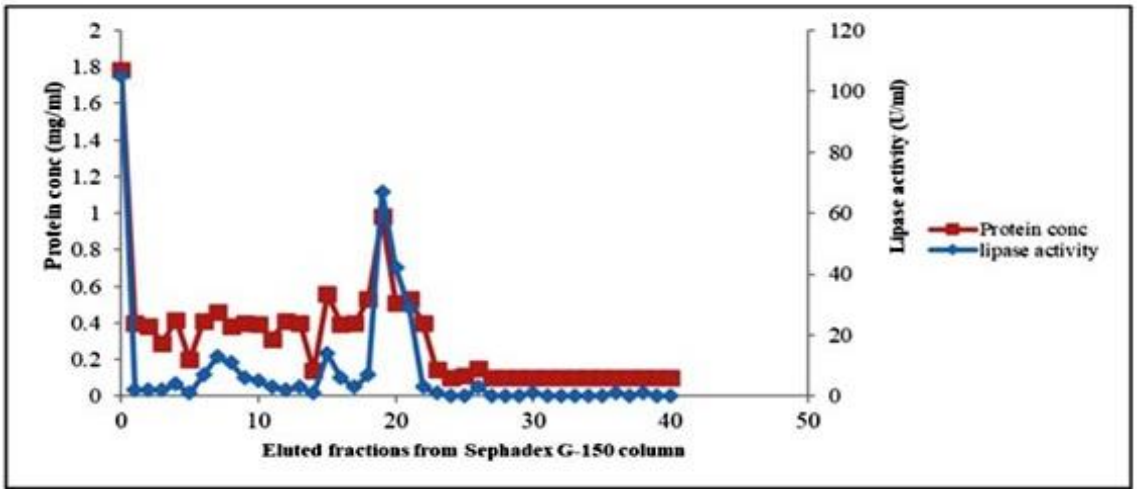


Fig 1: Chromatogram of Sephadex G-150 column chromatography.40 fractions of 5ml each were measured for Protein concentration and Enzyme activity.

Fig.2 Physical characterization of ELPL

Fig 2: Indicates the association of lipids in partially purified lipase shown by Thin-layer chromatography of lipids. Lane A: Partially purified lipase, Lane B: Olive oil.

Fig.3 Partially purified lipase at various purification stages

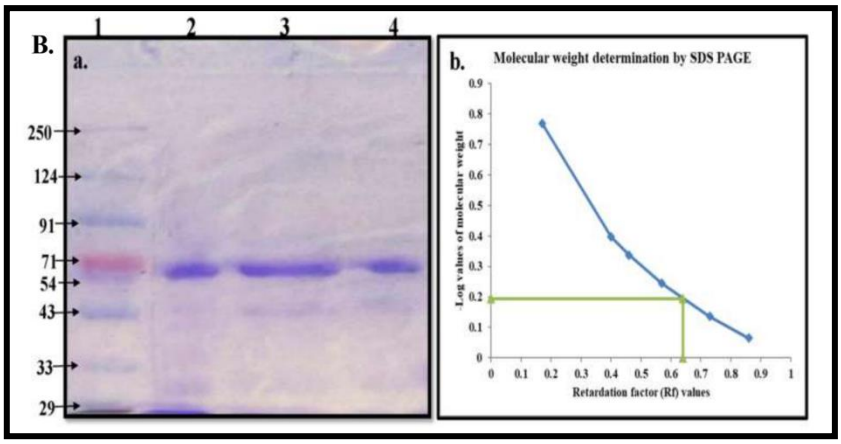


Fig 3: Indicates the partial purification of lipase at various stages by SDS-PAGE and molecular weight determination
a-10% SDS-PAGE indicates the partially purified lipase at various purification stages. Lane 1- Protein marker, Lane 2- Crude, Lane3 – Ammonium Sulfate precipitated fraction, Lane 4 – Pooled Sephadex G-150 fraction.
2b-Graph indicates the Molecular Weight determination of partially purified protein

Fig.4 Enzyme kinetics of partially purified ELPL

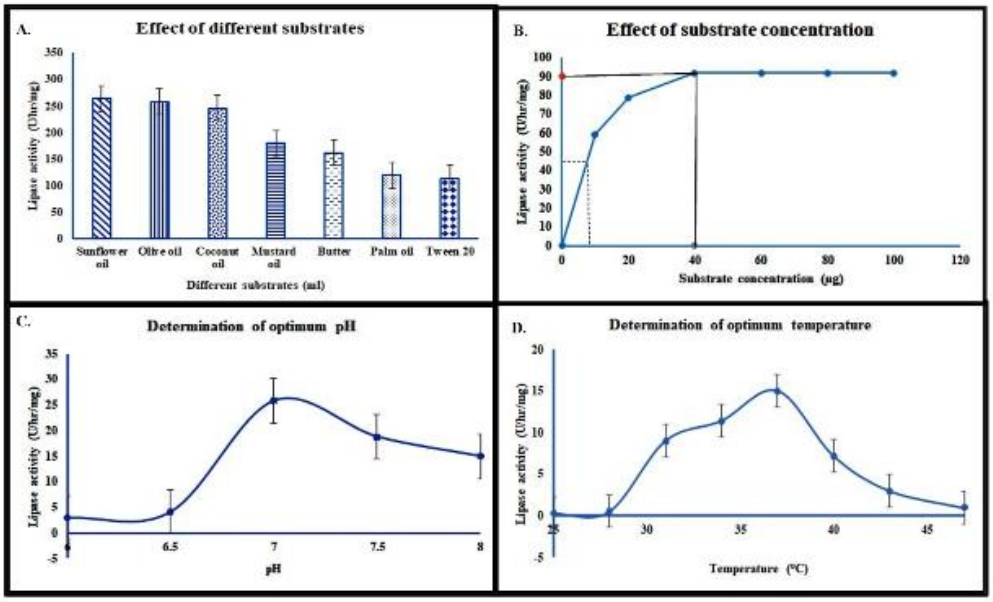
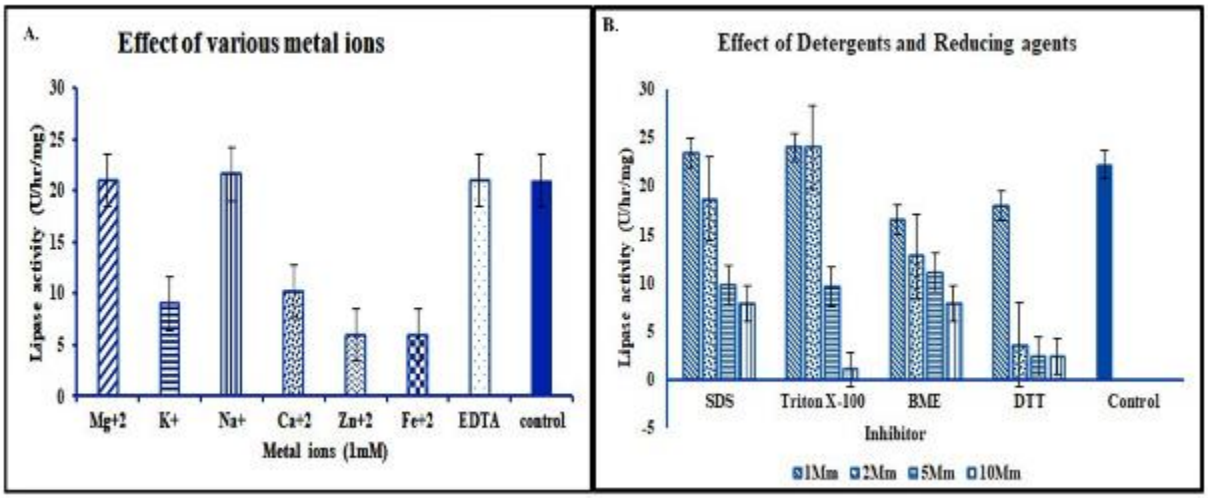


Fig 4:Indicates the effect of ELPL on different substrates, various substrate concentration, optimum pH, optimum temperature
A: Effect of ELPL in the hydrolysis of different substrates (Sunflower oil, Olive oil, Coconut oil, Butter Palm oil, Tween 20)
B: Effect of Substrate Concentration (10 µg to 120 µg) on partially purified ELPL.
C: ELPL activity at different pH ranges from pH 6 to 8.
D: ELPL activity at different temperature ranges between 25 ^{0}C to 45 ^{0}C
All the results represent the average of three independent experiments.

Fig.5 Effect of various metal ions, detergents and reducing agents on ELPL



A: Effect of various metal ions (Mg^{+2}, K^{+}, Na^{+}, Ca^{+2}, Zn^{+2}, Fe^{+2} and EDTA) on ELPL activity at 1mM concentration.
B: Effect of Detergents (Sodium Dodecyl Sulphate (SDS), Triton X-100), and Reducing agents (β-mercaptoethanol (BME), and Dithiothreitol (DTT)) on ELPL activity. The results represented are the averages of three independent experiments

Fig.6 Industrial applications of ELPL on dairy wastes

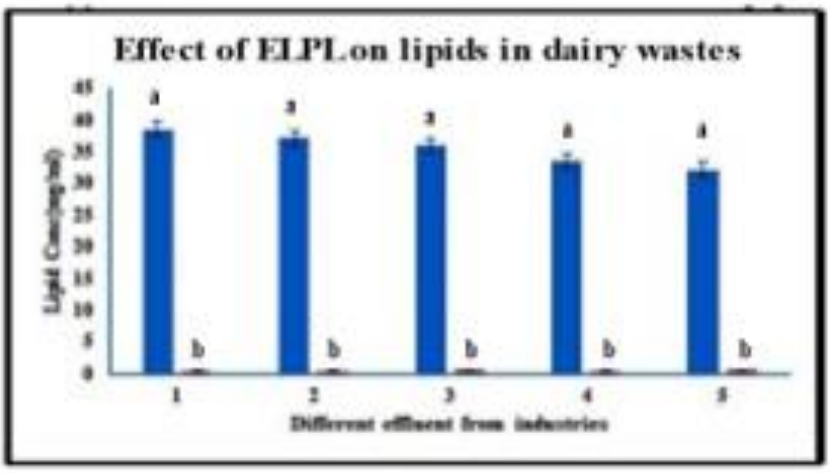


Fig 6: Indicates the degradation of the lipid content present in Dairy industry wastes.
a: Before treatment with ELPL b: After treatment with ELPL

Fig.7 Clinical applications of ELPL

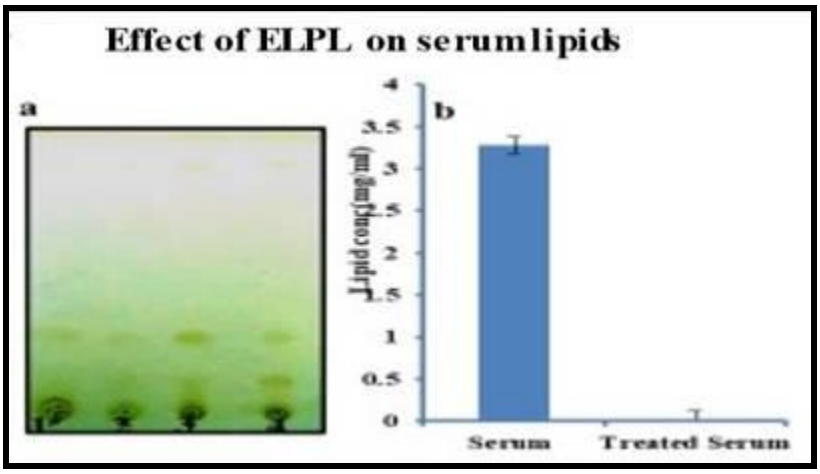


Fig 7: Indicates the hydrolysis of lipid content in serum.
A: The fig shows the Thin Layer Chromatography (TLC)
Lane 1&2: Before treatment with ELPL, Lane 3&4: After treatment with ELPL.
B: The fig shows the decrease in lipid content of serum by the Phosphovanillin method

Previously, a fungal lipase is produced from *Aspergillus niger* (Mhetras *et al.*, 2009; Nema *et al.*, 2019) by Solid Substrate Fermentation by using gingelly oil cake as a substrate (Muthazhagan and Thangaraj, 2014), however, lipase carried out in the current study was shown to be produced extracellularly by submerged fermentation process from the *Lysinibacillus fusiformis* L52, bacteria isolated from dairy industry wastes. It was also observed that other extracellular lipases were also produced from Pseudomonas spp., and *Serratia marcescens* (Sirisha *et al.*, 2010; Winkler and Stuckmann, 1979).

Extracellular lipase from *Lysinibacillus fusiformis* L52 is partially purified by using ammonium sulfate precipitation and column chromatography by using Sephadex G-150 and is purified to 4.39 folds with only 3.2% of yield. The previous report showed that lipase is partially purified from *G. stearothermophilus* AH22 strain by heat precipitation (700C, 30 min), DEAE-Cellulose Sephadex G-150, and Sephadex G-25 Gel permeation chromatography resulting in 18.3 folds purification with the yield of 19.7% (Arife Pınar Ekinci *et al.*, 2016). Whereas, a lipase from *Bacillus stearothermophilus* MC 7 showed a 10.2% yield with 19.25 folds and with a specific activity of 3.96 U/mg (Kaiquan Liu *et al.*, 2021). It was reported that 15.6-fold purification and 19.7% yield in the case of *Aneurinibacillus thermoaerophilus* HZ lipase by Q Sepharose and Sephadex G-75 chromatography.

Most of the lipases produced from bacteria are either glycoproteins or lipoproteins in nature (Tan *et al.*, 1999). The results based on lipid estimation and TLC of partially purified enzyme focus that the lipase obtained is lipoprotein in nature with has 0.08µg of lipids associated with 1mg of protein and named extracellular lipoprotein lipase (ELPL). Similarly, a lipase isolated from Rhizopus sp. is also found to be lipoproteinaceous in nature. Several reports suggested that most of the bacterial lipases abundant are either lipoprotein or glycoprotein in nature (Mobarak-Qamsari *et al.*, 2011). The molecular weight of partially purified ELPL is determined as 68.98 kDa, whereas acidic lipase purified from *Aspergillus niger* has a low molecular weight of 32.2 kDa (Mazhar *et al.*, 2017) and from *P. gessardii*, it is observed to be a high molecular weight with 94kDa (Jaganmai and Rajeswari, 2020).

Partially purified ELPL showed the optimum activity at pH 7.0, and at 37^0C with a Km value of

0.045mM by pNPP as substrate. It acts preferentially on plant-based lipids and has less intensity on animal fats like butter. It is also observed that ELPL specifically hydrolyses Olive oil and Sunflower oil, which contains basically a high percentage of unsaturated fatty acids like oleic acid and linolenic acid. Earlier it was also reported that lipase isolated from *Staphylococcus aureus* did not show any variations in hydrolyzing emulsions of coconut oil and serum activated coconut oil. Other lipases with neutral pH optimum were reported with *B. stearothermophilus* (Kaiquan Liu *et al.*, 2021), and several acidic and basic lipases from different bacterial species (Jaganmai and Rajeswari, 2020). The optimum temperature of ELPL, 37^0C varies from extracellular lipases derived from *A. niger* GZUF (36) and *Bacillus licheniformis* NCU CS-5, where they were observed at 35^0C and 50^0C successively (Vishnoi *et al.*, 2020).

The activity of ELPL did not have any effect when treated with Na^+, Mg^{+2}, and EDTA at 1mM Concentration, whereas K^+, Ca^{+2}, Zn^{+2}, Fe^{+2} ions cause the inhibition of 59%, 54%, 72%, and 72% respectively. Lipase derived from *B. ubonensis* SL-4 is activated by Ca^{+2} and Mn^{+2} ions, while Mg^{+2}, Co^{+2}, Cu^{+2}, Pb^{+2}, Al^{+3}, and Fe^{+3} strongly inhibit its activity as per the reports. Ca^{+2} and Mg^{+2} promote the activity of a lipase from *Serratia liquefaciens* (Winkler and Stuckmann, 1979), while Zn+2 and Fe+2are both inhibitory factors (Arife Pınar Ekinci *et al.*, 2016).

It was reported that the extracellular lipase produced by *B. stearothermophilus* MC 7 was inhibited by the presence of divalent ions like Cu^{+2} and Zn^{+2} in the reaction mixture, while the enzyme remained completely active in the presence of Ca^{+2} ions, but the partially purified lipoprotein lipase is Ca^{+2} independent as it did not show any effect with EDTA. Hence it can be concluded that the partially purified lipase is metal independent enzyme.

When analyzed for the effect of detergent-like SDS, surfactant like Triton X-100, and thiol reducing agents like DTT & β-ME upon ELPL activity, strong inhibition was observed at 10mM concentration, speculating that the enzyme is a lipoprotein containing disulfide bonds in its structure. In contrast, it was reported previously that lipase derived from *B. ubonensis* SL-4 is activated by Tween-20, Tween-40, Tween-60, Tween-80, Triton X-100, but inhibited by EDTA, SDS, DTT, and β-Merkaptoethanol (Enrique and Herrera-López, 2012).

The partially purified ELPL has shown the ability to hydrolyze lipids (Fig 6 B) in the serum and also lipids present in the dairy industry effluents (Fig 6A). Thus, the partially purified ELPL can be used for Industrial and Clinical applications. Already it was reported that the lipases isolated from microbes like *C. rugosa, Pseudomonas, Bacillus, Acinetobacter*, etc. are involved in the digestion of skimmed fat-rich effluents (Jaganmai and Rajeswari, 2017).

Thus the lipases from different microbial sources are mostly used in the treatment of industrial wastewater effluents like dairy, food, wool, and oil mills (Chandra *et al.*, 2020). Lipases have a wide variety of pharmaceutical and clinical applications (Dougherty *et al.*, 1991).

The lipoprotein lipases play a key role in clinical applications, especially in the catalysis of plasma triglycerides (Rajeswari *et al.*, 2009). Even pathogenic bacteria like P. acnes, Corynebacterium acnes, and *Staphylococcus aureus* lipases have been initiated to have an influence on skin rash in acne patients18 and also as a potential candidate for cancer treatment (Takasu *et al.*, 2012).

Lipase produced from *Lysinibacillus fusiformis* L-52 bacteria isolated from dairy industry wastes was partially purified showing a significant effect in minimizing the major lipid pollutant present in the effluents of dairy industries. And it can also be concluded that lipase produced from isolated bacteria present in dairy industry wastes has shown a significant effect in minimizing the total lipids present in the serum samples.

In which the lipids were extensively hydrolyzed. The partially purified lipoprotein lipase has effect in hydrolyzing the lipid pollutant present in the effluents of dairy industry and also observed that there is significant decrease in total lipids present in serum. Hence the partially purified lipase obtained from dairy industrial wastes has effectively hydrolysed the lipid pollutants both in serum and industrial effluents.

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