Purification and characterization of lipase from a new strain *Staphylococcus argenteus* MG2 (MTCC 12820)

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Abstract

The lipase enzyme was isolated and purified from *Staphylococcus argenteus* MG2 (MTCC 12820) to homogeneity using ammonium sulphate precipitation followed by chromatographic techniques. This process resulted in a purification factor of 40.96-fold and a 26.25% recovery with a specific activity of 744.68 U mg^{-1}. The molecular weight of the purified lipase was determined by SDS-PAGE to be 45 kDa. The Km and Vmax values of the purified lipase were calculated to be 4.95 mM and 79.36 µmol/min/mg^{-1}, respectively. The maximum lipase activity was observed at pH 7.0 and 30 ºC with 100% stability, and it was also found to be stable in a broad range of pH (5-12) and temperature (30-90 ºC). The enzymatic activity of this *Staphylococcal* lipase was increased by Ca^{2+} to 105.71% at a concentration of 1 mM $CaCl_2$. Additionally, it exhibited marked stability and activity in organic solvents. In the presence of 1% SDS surfactant, it retained 85.16% residual activity, while the metal chelator EDTA (inhibitor) reduced the lipase activity to 83.87% residual activity at a concentration of 1% *w/v*. This alkali-stable and thermo-stable lipase can be exploited by extending its use in the preparation of detergents and in various industrial and biotechnological applications.

Keywords: *Staphylococcus argenteus* MG2, lipase, purification, characterization.

Purificação e caracterização da lipase de uma nova cepa - *Staphylococcus argenteus* MG2 (MTCC 12820)

Resumo

A enzima lipase foi isolada e purificada de *Staphylococcus argenteus* MG2 (MTCC 12820) até à homogeneidade utilizando precipitação com sulfato de amônio seguida por técnicas cromatográficas. Esse processo resultou em um fator de purificação de 40,96 vezes e uma recuperação de 26,25% com uma atividade específica de 744,68 U mg^{-1}. O peso molecular da lipase purificada foi determinado por SDS-PAGE como sendo de 45 kDa. Os valores de Km e Vmax da lipase purificada foram calculados como sendo de 4,95 mM e 79,36 µmol/min/mg^{-1}, respectivamente. A atividade máxima da lipase foi observada em pH 7,0 e 30 ºC com 100% de estabilidade, e foi encontrada para ser estável em uma ampla faixa de pH (5-12) e temperatura (30-90 ºC). A atividade enzimática desta lipase estafilocócica foi aumentada pelo Ca^{2+} para 105,71% a uma concentração de 1 mM de $CaCl_2$. Além disso, ela exibiu estabilidade e atividade marcadas em solventes orgânicos. Na presença de 1% de surfactante SDS, ela reteve 85,16% da atividade residual, enquanto o quelante de metal EDTA (inibidor) reduziu a atividade da lipase para 83,87% de atividade residual a uma concentração de 1% *p/v*. Esta lipase, estável tanto em meio alcalino quanto termoestável, pode ser explorada ao estender seu uso na preparação de detergentes e em diversas aplicações industriais e biotecnológicas.

Palavras-chave: *Staphylococcus argenteus* MG2, lipase, purificação, caracterização.

1. Introduction

Some extracellular bacterial lipases are lipoproteins, while others are glycoproteins. Among bacteria, various

species such as *Achromobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Chromobacterium* sp. have been utilized for the production of lipases (Godfredson et al., 1990). Many Staphylococci are capable of producing extracellular lipases, and several of them have been isolated, purified, and extensively studied for their biochemical properties (Oort et al., 1989; Gotz et al., 1985; Farrell et al., 1993; Lee; Iandolo, 1986; Talon et al., 1996; Oh et al., 1999; Simons et al., 1998; Jaeger et al., 1999; Van-Kampen et al., 2001; Pandey et al., 1999).

In order to maximize lipase yields, it is crucial to consider optimal growth conditions (Linefield et al., 1990). Bacterial lipases, primarily extracellular, are significantly influenced by various nutritional and physicochemical factors such as pH, temperature, carbon and nitrogen sources, inorganic salts, presence of lipids, dissolved oxygen concentration, and agitation (Brune; Gotz, 1992; Aires-Barros et al., 1994; Jeager et al., 1994; Kim et al., 1996).

Various methods of lipase assay have been classified, including titrimetry, interfacial tensiometry, spectroscopy, chromatography, immunochemistry, and conductimetry (Beisson et al., 2000; Kulkarni, 2002). Among these, titrimetry stands out as the simplest method, albeit requiring continuous pH meter readout, known as the pH stat method. For purification, techniques such as precipitation, hydrophobic interaction chromatography, gel filtration, ion exchange chromatography, and affinity chromatography are commonly employed (Woolley; Peterson, 1994).

For instance, lipases from various *Pseudomonas* species were purified through acidification of culture supernatant, ammonium sulfate precipitation, sepharose CL-6B chromatography, and isoelectric focusing. Purified lipases from *Pseudomonas fragi, Pseudomonas fluorescens,* and *Pseudomonas aeruginosa* were found to be monomeric. The lipase gene of *P. fragi* has been cloned and sequenced (Saxena et al., 1999). Additionally, a lipase (29 kDa) from *P. aeruginosa* MB5001 was purified (Chartrain et al., 1993).

Staphylococcus epidermidis RP 62A's lipase enzyme was purified using precipitation techniques, affinity chromatography, and gel filtration. The enzyme's optimum pH was found to be 6.0, requiring calcium as a cofactor for catalytic activity (Simons et al., 1998). A thermostable lipase (45 kDa) produced by a thermophilic Bacillus sp. J33 was purified to 175-fold using ammonium sulfate and phenyl sepharose column chromatography (Nawani; Kaur, 2000).

In another study, the apparent Michaelis–Menten constants (Km) for immobilized and free lipase produced by *Pseudomonas pseudomallei* 12Sm were reported as 555 and 178 µM, respectively. The catalytic efficiency of the lipase (Kcat) did not significantly alter upon immobilization in the polyvinyl alcohol (PVA) membrane (PAM). The activity of the immobilized lipase increased by up to 4.3-fold at a 0.2% water concentration in the reaction mixture and this enhancement was sustained up to the fifth reaction cycle (Kanwar et al., 2002).

Lipases have emerged as pivotal products in the rapidly growing biotechnology industry due to their versatile applications. Given the importance of lipase enzymes, this research aims to purify and characterize lipase from *Staphylococcus argenteus* MG2.

2. Materials and Methods

2.1 Purification of lipase

Purification of lipase was performed by ammonium sulphate precipitation, dialysis, ion exchange chromatography, gel filtration and SDS- PAGE method.

2.1.1 Separation of crude lipase enzyme

Staphylococcus argenteus MG2, isolated from vegetable oil-contaminated soil in Indore, Madhya Pradesh, exhibited significant lipase activity, producing a zone of hydrolysis (2.57 mm) on Tributyrin agar. It showed optimal growth and lipase activity (3.12 mm) at pH 9, indicating alkali tolerance. Identified at the species level using 16S rDNA sequencing, MG2 was deposited with MTCC under accession number MTCC 12820, and its sequence was recorded in the NCBI Gene Bank (KY082046). Lipase activity was measured titrimetrically, affirming its enzymatic potential (Golani et al., 2019).

The culture of the selected lipolytic bacterial isolate *Staphylococcus argenteus* MG2 (Golani et al., 2019) was centrifuged at 4 ºC and 10,000 rpm for 30 min. The cell-free supernatant was then utilized as crude extracellular lipase. Lipase purification was carried out following the method of Hiol et al. (2000) with certain modifications.

2.1.2 Ammonium sulfate precipitation

Ammonium sulfate was gradually added to the chilled cell-free supernatant to achieve 60% (*w/v*) saturation at 4 ºC. The precipitate formed after standing in the ammonium sulfate solution for 4 h was separated by centrifugation at 4 ºC and 10,000 rpm for 30 min. The pellets obtained were re-dissolved in 10 mL of 0.05 M phosphate buffer (pH 7.2) and dialyzed overnight against 2 L of the same buffer.

2.1.3 Ion exchange chromatography

The dialyzed enzyme sample was applied to a DEAE Cellulose (Sigma-Aldrich, USA) column pre-equilibrated with 0.01 M phosphate buffer, pH 7.2. The bound protein was eluted with a linear gradient of NaCl (0.1-0.5 M) in the same buffer. The fractions of protein content were measured by taking the optical density at 280 nm. The protein-containing fractions were then assayed for lipase activity.

2.1.4 Gel filtration chromatography

The dialyzed enzyme solution was loaded onto a Bio-Gel P-100 column previously equilibrated with 0.01 M phosphate buffer (pH 7.2), containing 0.15 M NaCl. Elution was carried out with the same buffer at a flow rate similar to that of blue dextran, i.e., 0.30 mL/min at 7-10 ºC, and 50 fractions of 3 mL each were collected. These fractions were subsequently assayed for lipase activity and estimation of protein content. The active fractions obtained were pooled and used to determine the molecular mass of the native enzyme.

2.2 Molecular Characterization

2.2.1 Molecular weight determination of lipase by SDS-PAGE analysis

The molecular weight of the lipase was determined via SDS-PAGE analysis following the method of Laemmli (1970) using standard protein markers: α-Lactalbumin (14.3 kDa), Trypsin inhibitor (20.1 kDa), Carbonic anhydrase (29 kDa), IgG (50 kDa), Bovine serum albumin (66 kDa), and Phosphorylase B (97.4 kDa). The SDS-PAGE analysis (Bio-Rad, CA) was conducted with a resolving gel of 12% acrylamide and a stacking gel of 4.5% acrylamide.

After the gel run, it was washed with distilled water and immersed for 15 min in a fixative solution (30% ethanol, 10% acetic acid, 0.05% formalin). Subsequently, the gel was stained for 2 h in Coomassie Brilliant Blue R 250 staining solution. Excess stain on the gel was removed to reveal the bands by placing the gel in a destaining solution. After several destaining steps, the gel was stained with 0.2% *w(/v*) Silver nitrate, 0.076% formalin solution for 10-15 min. Following a brief wash, the gel was developed with 100 mL of developer solution (6% *w/v* Na_2CO_3, 0.05% formalin) until the bands appeared.

2.2.2 Detection of lipase activity of purified lipase on tributyrin agar medium plate

10 µL of the purified lipase enzyme sample obtained after DEAE cellulose column chromatography was placed on a 2% agar plate containing tributyrin (1%, *w/v*). The plate was then incubated at 37 ± 2 ºC for 24 h, during which the hydrolysis of tributyrin revealed the location of the enzyme.

2.3 characterization of lipase

2.3.1 Characterization of enzymatic activity of lipase

The characterization of the purified lipase obtained after DEAE cellulose column chromatography was conducted to assess stability in pH, temperature, effects of metal ions, organic solvents, surfactants, inhibitors, and with different enzyme substrates. All experiments were carried out using the titrimetric method (Macedo, 1997).

2.3.1.1 Effect of substrate concentration on lipase activity

The activity of the lipase towards different concentrations of olive oil (0-30 mM) as a substrate was determined by pH-stat assay under optimal conditions (pH 7.2 and 37 ºC). The apparent Michaelis-Menten constant (Km app) value and Vmax (maximum velocity) were determined graphically from double reciprocal Lineweaver-Burk plots of the kinetic data. Additionally, the Km value and Vmax of commercial lipase (Lipoprotein lipase from *Pseudomonas* sp.) were studied under the same experimental conditions for comparison.

2.3.1.2 Effect of pH on lipase activity

The pH stability of the lipase was investigated in the pH range of 2.0 to 12.0. Buffers used were acetate buffer for pH 4 and 5, phosphate buffer for pH 6, 7, and 8, and glycine-sodium hydroxide buffer for pH 9 and 10.

The lipase was incubated in buffer solutions of respective pH at 37 ºC for 2 h. Relative activity was measured as the ratio of activity obtained after pre-incubation at a certain pH to the activity obtained after pre-incubation at the pH exhibiting maximum lipase activity in the investigated range of pH, expressed as a percentage (Castro-Ochoa et al., 2005).

2.3.1.3 Effect of temperature on lipase activity

The thermostability of the lipase was determined by incubating the enzyme in phosphate buffer (0.05 M, pH 7.2) at different temperatures (20-90 ºC) under standard assay conditions.

2.3.1.4 Effect of metal ions on lipase activity

The lipase enzyme was incubated in respective metal salts ($MgSO_4$, $MnSO_4$, $CuSO_4$, $FeSO_4$, $CaCl_2$, NaCl, and $ZnSO_4$) at 37 ºC for 2 h to measure relative activity.

2.3.1.5 Effect of organic solvents on lipase activity

The lipase enzyme was incubated in a 50% (*v/v*) solution of different organic solvents (acetone, methanol, and isopropanol) in phosphate buffer to measure relative activity, evaluating the effect of organic solvents on lipase stability.

2.3.1.6 Effect of surfactants on lipase activity

For evaluating the effect of surfactants on lipase, 2 mL of lipase was incubated in 2 mL of various surfactants (SDS, Tween 80, and Triton X-100) in phosphate buffer (0.05 M, pH 7.2) for 2 h at 37 ºC (Rathi et al., 2001). Subsequently, 0.2 mL aliquots of the mixtures were taken to measure lipase activity under standard assay conditions using the titrimetric and spectrophotometric methods (Equation 1).

$$\text{Lipase activity (\%)} = \frac{\text{Activity of control} - \text{Activity of treated sample}}{\text{Activity of control}} \times 100 \quad \text{Eq. (1)}$$

2.3.1.7 Effect of inhibitors on lipase activity

The purified lipase was incubated in solutions of various inhibitors, EDTA, $HgCl_2$ (1%, *w/v*) and β-mercaptoethanol (1%, *v/v*) in phosphate buffer (0.05 M, pH 7.2) for 2 h at 37 ºC to measure residual activity under standard assay conditions.

3. Result

3.1 purification of lipase

The crude lipase enzyme was purified in several steps with increase in its purity of specific activity.

3.1.1 Separation of crude lipase enzyme

The cell-free supernatant of *S. argenteus* MG2 was utilized as the crude extracellular lipase source. Lipase purification followed the method outlined by Hiol et al. (2000) with specific adjustments.

3.1.2 Ammonium sulfate precipitation

In this study, the crude enzyme extracts from isolate *S. argenteus* MG2 displayed a gradual increase in lipase yield up to 60% saturation of ammonium sulfate. The ammonium sulfate precipitated lipase enzyme (60%) underwent overnight dialysis against 2 L of 0.05 M phosphate buffer (pH 7.2) to eliminate excess ammonium sulfate. The optical density (OD) of the dialyzed enzyme was measured at 280 nm, yielding a value of 1.414. The concentration of total protein was determined to be 0.57 mg mL^{-1}.

3.1.3 Ion exchange chromatography

A 3 mL portion of the dialyzed lipase enzyme sample was introduced into a DEAE cellulose column that had been pre-equilibrated with sodium phosphate buffer (0.01M, pH 7.2), allowing it to traverse the column. The bound enzyme was subsequently eluted using sodium phosphate buffer (0.01M, pH 7.2) with a linear gradient of NaCl (ranging from 0.1 to 0.5 M). Each concentration of NaCl (10 mL solution) was employed to elute the bound enzyme. The protein content of the fractions was assessed by measuring OD at 280 nm. Lipase activity was detected in the fraction released upon addition of 0.1M NaCl (Figure 1).

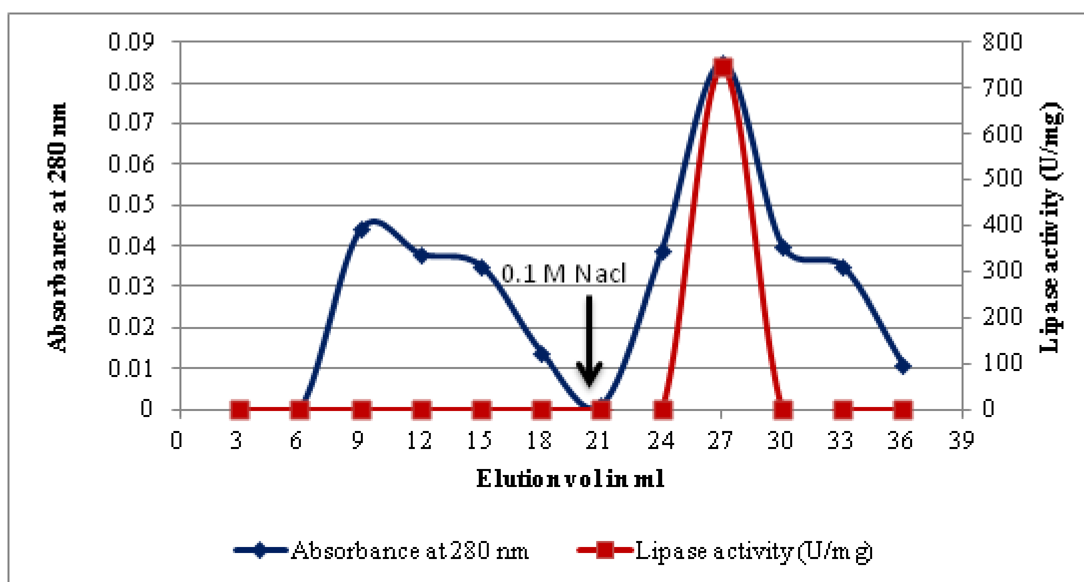

Figure 1. Chromatographic purification profile of extracellular lipase on DEAE cellulose column chromatography. Source: Authors, 2023.

The purification flow sheet is shown in (Table 1). The 40.96 fold purification factor was obtained during purification process with a final recovery of 26.25% of the enzyme with specific activity 744.68 U mg^{-1}. Lipase enzyme bound to DEAE cellulose was eluted with 0.1 M NaCl.

Table 1. Purification summary of lipase from *Staphylococcus argenteus* MG2.

Purification step	Total enzyme activity (U mL^{-1})	Total protein (mg)	Specific activity (U mg^{-1})	Recovery (%)	Purifi-cation (fold)
Culture supernatant	400	22	18.18	100	1
Ammonium sulphate precipitation (60%)	216.6	1.49	145.36	54.15	7.99
DEAE cellulose column	105	0.14	744.68	26.25	40.96

Source: Authors, 2023.

3.1.4 Gel filtration chromatography

The dialyzed enzyme solution was applied to a Bio gel P-100 column previously equilibrated in 0.01 M phosphate buffer (pH 7.2), containing 0.15 M NaCl. The void volume of the column was determined using blue dextran and was found to be 40 mL. Figure 2 shows the fractionation pattern of lipase on Bio gel P-100 column. One distinctive protein peak appeared that overlapped with the lipase activity which was used to determine the molecular mass of the native enzyme. The total volume of the column was determined to be 159.19 cm^3. For the determination of molecular weight, the gel filtration data of marker proteins was analyzed by the method of least square to obtain the following equation:

$$Ve/Vo = -1.4465 \times \text{Log}_{10}M + 8.097$$

Where M is the molecular weight

The linear plot according to the above equation is depicted in (Figure 2). The values of Ve/Vo and $\text{Log}_{10}M$ for marker proteins and lipase enzyme are given in (Table 2). The value of Ve/Vo of 1.40 for pure lipase enzyme corresponds, according to (Figure 3) to a molecular weight of 47 kDa.

Table 2. Values of molecular weight, Ve/Vo, $log_{10}M$ of the marker proteins and lipase enzyme of *Staphylococcus argenteus* MG2 in gel filtration experiments.

S. No.	Proteins	Molecular weights (kDa)	Ve/Vo	$Log_{10}M$
1	Bovine serum albumin	67.0	1.18	4.8260
2	Ovalbumin	43.0	1.40	4.6334
3	α- chymotrypsinogen A	25.0	1.54	4.3997
4	Ribonuclease A	13.7	2.11	4.1367
5	Cytochrome C	12.4	2.205	4.073
6	Lipase enzyme of *Staphylococcus argenteus* MG2	47.0	1.40	4.650

Source: Authors, 2023.

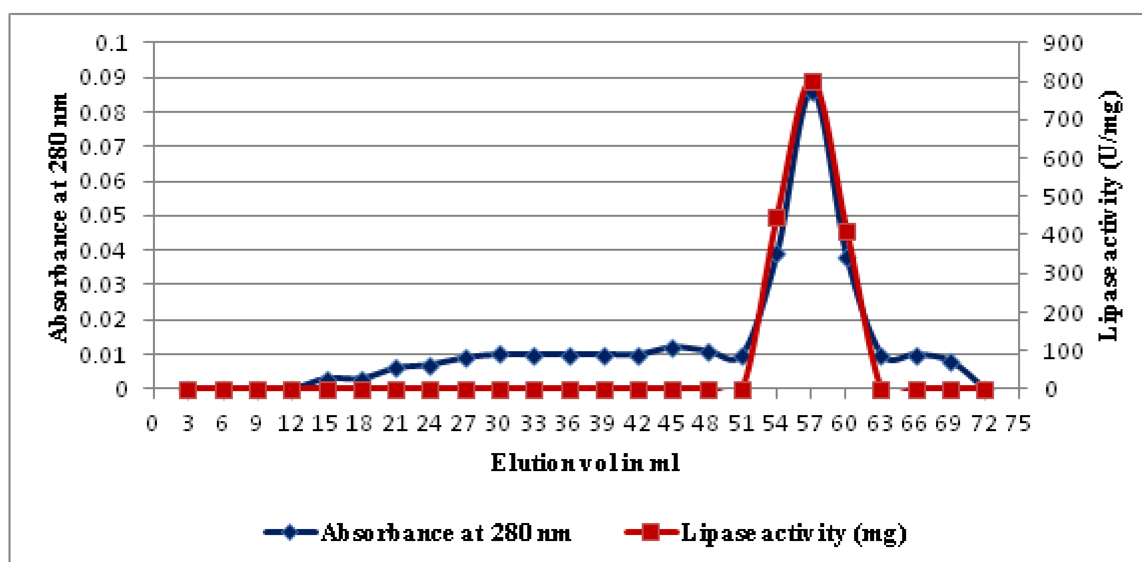

Figure 2. Elution profile of extracellular lipase on Bio gel P-100 gel filtration chromatography. Source: Authors, 2023.

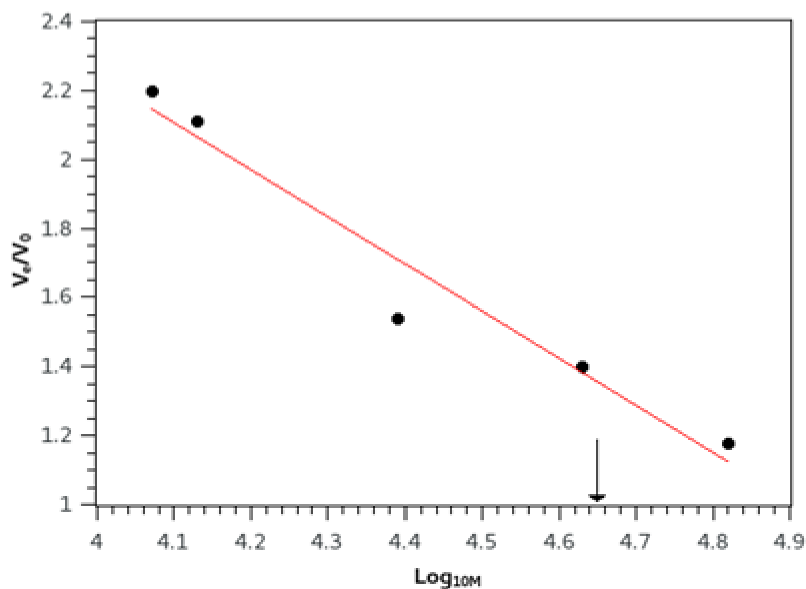

Figure 3. Andrew's plot of Ve/Vo versus $Log_{10}M$. Source: Authors, 2023.

The marker proteins used were (1) bovine serum albumin, (2) ovalbumin, (3) α-chymotrypsinogen A, (4) ribonuclease A and (5) cytochrome C. The value of $Log_{10}M$ for lipase enzyme of *S. argenteus* MG2 is shown by an arrow. The linear plot was obtained according to Andrew (1970), by the method of least square and fits the equation:

$$Ve/Vo = -1.4465 \times Log_{10}M + 8.097$$

3.2 Molecular characterization

3.2.1 Molecular weight determination of lipase by SDS-PAGE analysis

The purified lipase was found to be homogenous when tested by SDS-PAGE (Figure 4 lane 3). The purity of the lipase enzyme was confirmed by the presence of a single band on SDS-PAGE with a molecular mass of approximately 45 kDa. For the determination of molecular weight, the SDS-PAGE data was analyzed by the method of least square to obtain the following equation:

$$Log_{10}M = -0.9545 \times Rm + 4.8631$$

Where M is the molecular weight

The linear plot according to the above equation is depicted in (Figure 5). The values of Rm and $Log_{10}M$ for marker proteins and lipase enzyme are given in (Table 3).

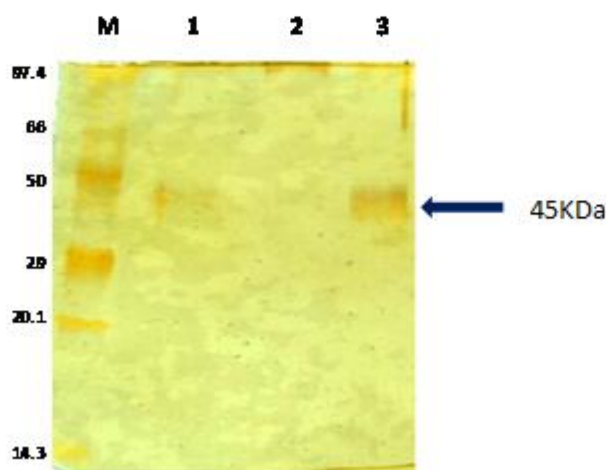

Figure 4. SDS-PAGE of purified lipase from *Staphylococcus argenteus* MG2. Source: Authors, 2023.

Lane- M: Molecular weight marker

Lane – 1 and 3: purified lipase enzyme stained with silver stain (45kDa)

The value of Rm of 0.869 for pure lipase enzyme corresponds, according to (Figure 5) to a molecular weight of 45 kDa.

Table 3. Molecular weight and relative mobility (Rm) for marker proteins and lipase enzyme of *Staphylococcus argenteus* MG2 used in SDS-PAGE.

S. No.	Proteins	Molecular weight (kDa)	$Log_{10}M$	Rm
1	Phosphorylase B	97.4	4.9885	0.783
2	Bovine serum albumin	66.0	4.8195	0.811
3	IgG	50.0	4.6989	0.832
4	Carbonic anhydrase	29.0	4.4623	0.876
5	Trypsin inhibitor	20.1	4.3031	0.908
6	α-Lactalbumin	14.3	4.1553	0.940
7	Lipase enzyme of *Staphylococcus argenteus* MG2	45.0	4.6500	0.869

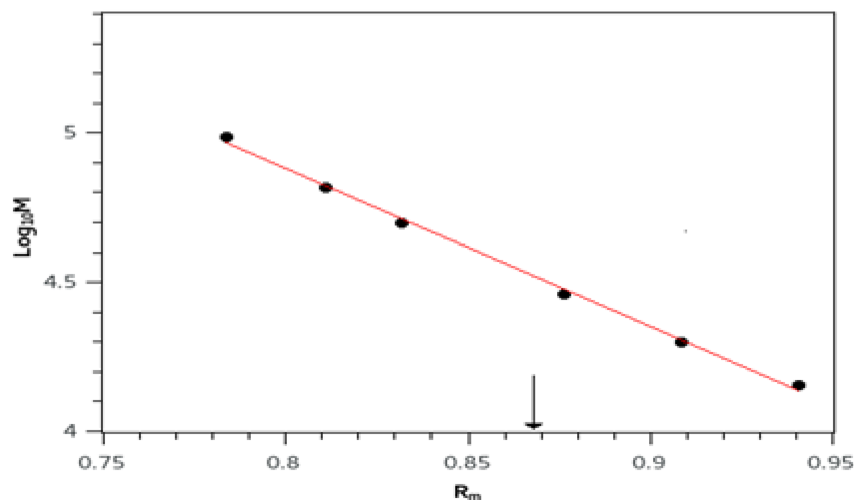

Figure 5. Plot of Rm values of marker proteins versus logarithms of molecular weight. Source: Authors, 2023.

The marker proteins used were (1) Phosphorylase B (97.4 kDa); (2) Bovine serum albumin (66 kDa); (3) IgG (50 kDa); (4) Carbonic anhydrase (29 kDa); (5) Trypsin inhibitor (20.1 kDa); (6) α-Lactalbumin (14.3 kDa). The $Log_{10}M$ value of the lipase enzyme of *Staphylococcus argenteus* MG2 is indicated by an arrow. The marker proteins were electrophoresed under denaturing conditions as described in Materials and Methods. The straight line obtained, by the help of least square analysis, fits the equation: $Log_{10}M = -0.9545 \times Rm + 4.8631$.

3.2.2 Detection of lipase activity of purified lipase on tributyrin agar medium plate

10 µL of the purified lipase enzyme sample obtained after DEAE cellulose column chromatography hydrolyzed tributyrin which was placed on 2% agar plate containing tributyrin (1%, *w/v*) (Longshaw et al., 2000) . Clear zone of tributyrin hydrolysis showed the location of the enzyme and confirms its lipase activity also (Figure 6).

Figure 6. Detection of lipase activity of purified lipase from *Staphylococcus argenteus* MG2 on tributyrin agar medium plate. Source: Authors, 2023.

3.3 Characterization of lipase

3.3.1 Characterization of enzymatic activity of lipase

Characterization of purified lipase enzyme activity was investigated.

3.3.1.1 Effect of substrate concentration on lipase activity

Lipase activity increased with increasing olive oil substrate concentration from 3 mM to 12 mM. At a substrate concentration of 12 mM, lipase activity reached 100%, and it remained consistently linear up to a substrate concentration of 21 mM (Figure 7). Lipases constitute a family of enzymes capable of independently hydrolyzing long-chain triacylglycerols. The lipase produced by *S. argenteus* MG2 demonstrates genuine lipase activity, efficiently hydrolyzing olive oil.

Determination of Km Value of Lipase

The kinetic parameters of the lipase produced by *S. argenteus* MG2 were further investigated by determining the rates of hydrolysis at various concentrations of olive oil. Analysis of the Lineweaver-Burk plot (Figure 8) yielded an apparent substrate affinity constant (Km app) of 4.95 mM and a maximal velocity value (Vmax) of 79.36 µmol/min/mg under standard assay conditions. In comparison, the Km app of commercial lipase (Lipoprotein lipase from *Pseudomonas* sp.) was determined to be 3.22 mM, with a corresponding Vmax of 50 $\mu mol/min/mg^{-1}$ under standard assay conditions.

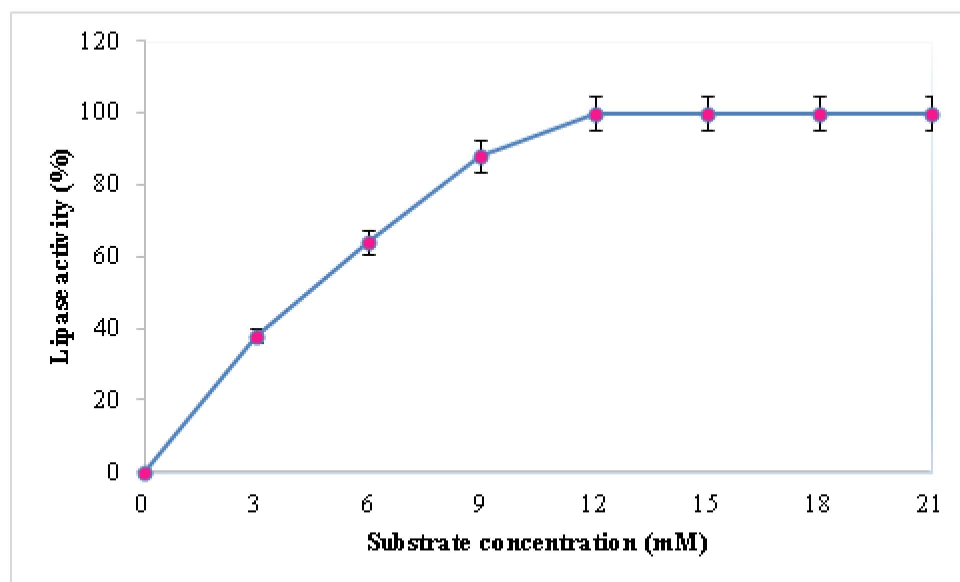

Figure 7. Effect of substrate concentration on lipase activity. Source: Authors, 2023.

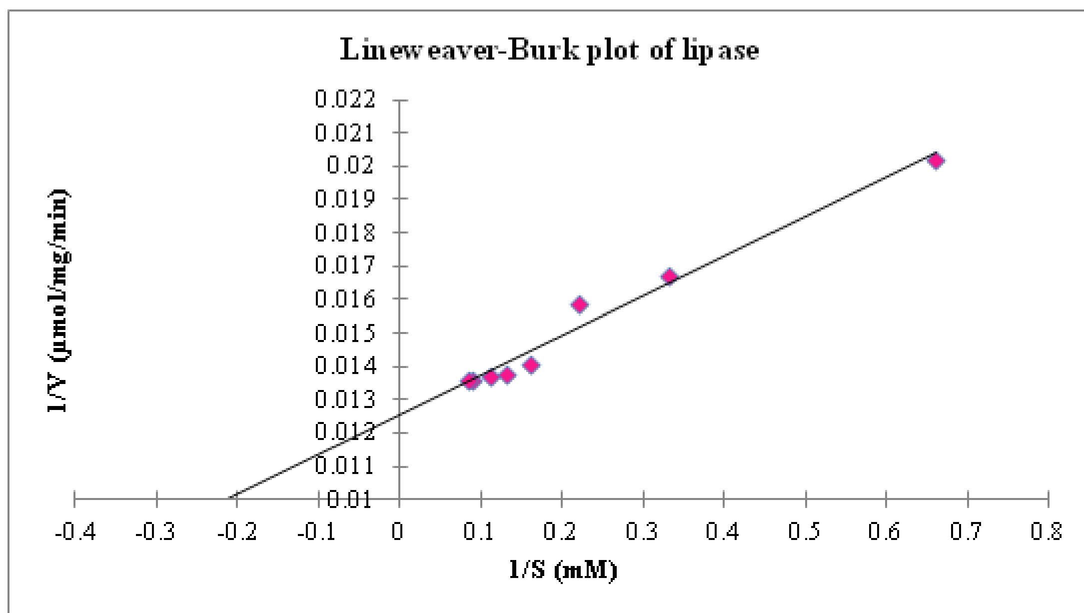

Figure 8. Lineweaver-Burk plot of lipase. Source: Authors, 2023.

3.3.1.2 Effect of pH on lipase activity

The emphasis of research work currently being done on lipases is to identify and develop lipases stable and working in alkaline conditions which could be used as fatty stain remover. Each enzyme has an optimum pH for most efficient function. In the present study, the activity profile of the lipase production by *S. argenteus* MG2 at different pH showed that the lipase is stable at a broad range of the pH values between pH 5-12 (Figure 9).

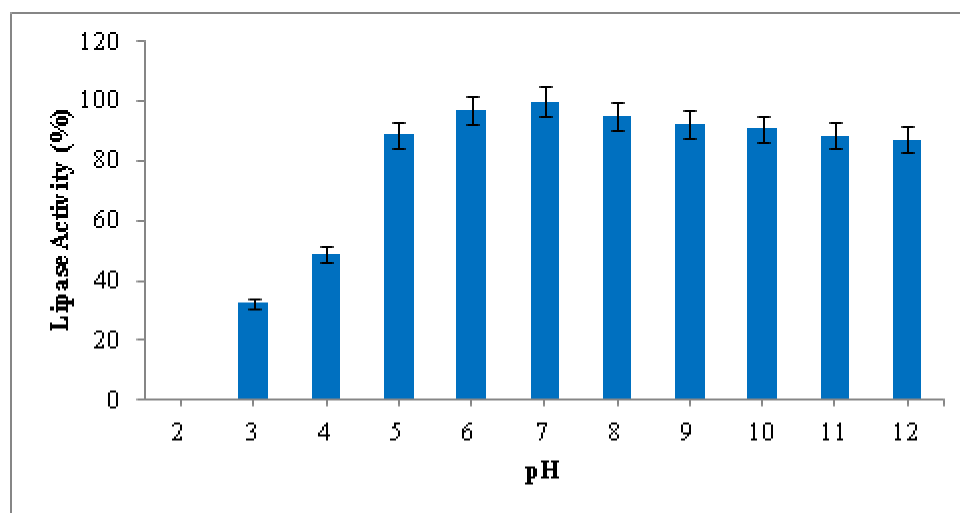

Figure 9. Effect of pH on lipase activity. Source: Authors, 2023.

3.3.1.3 Effect of temperature on lipase activity

The lipase activity profile was also studied by performing the enzyme assay at different temperatures between 20-90 ºC. 80% of lipase activity was observed at 20 ºC. The optimum temperature of lipase obtained from *S. argenteus* MG2 was 30 ºC with 100% lipase activity. The lipase activities decreased to 94.22, 93.33, 91.11 and 88.88% respectively at temperatures of 40 ºC, 50 ºC, 60 ºC, and 70 ºC. Then it became stable up to 90 ºC. Various *Staphylococcus* sp. were found to be stable at high temperature ranges (Figure 10).

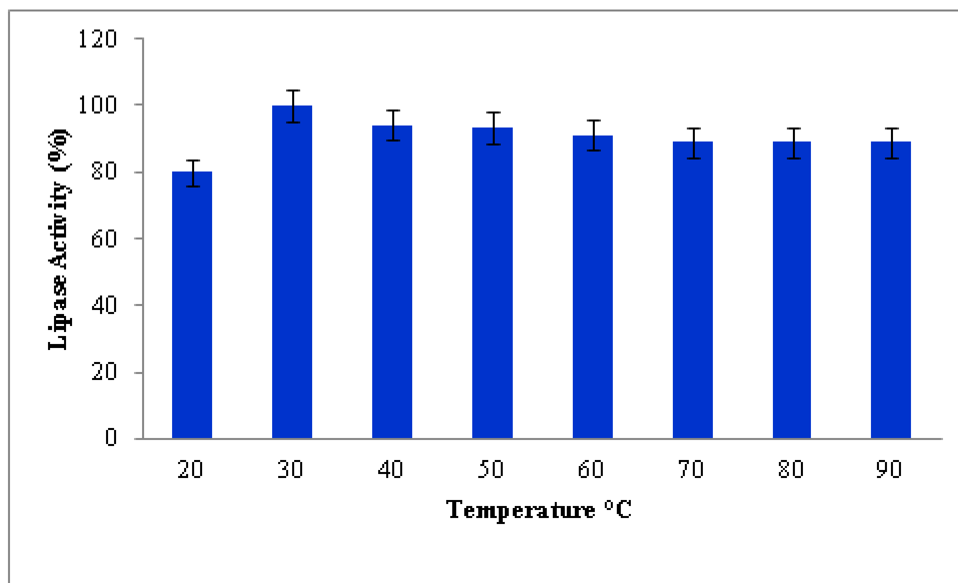

Figure 10. Effect of temperature on lipase activity. Source: Authors, 2023.

3.3.1.4 Effect of metal ions on lipase activity

The results of metal ions tested have been shown in (Figure 11). Among the metal ions tested, Ca^{2+} ions enhanced the lipase activity with 105.71% while Mg^{2+} ions had no significant effect. Mn^{2+}, Cu^{2+}, Fe^{2+}, Na^{+}, Zn^{2+} ions reduced the enzyme activity which indicates the competitive inhibition.

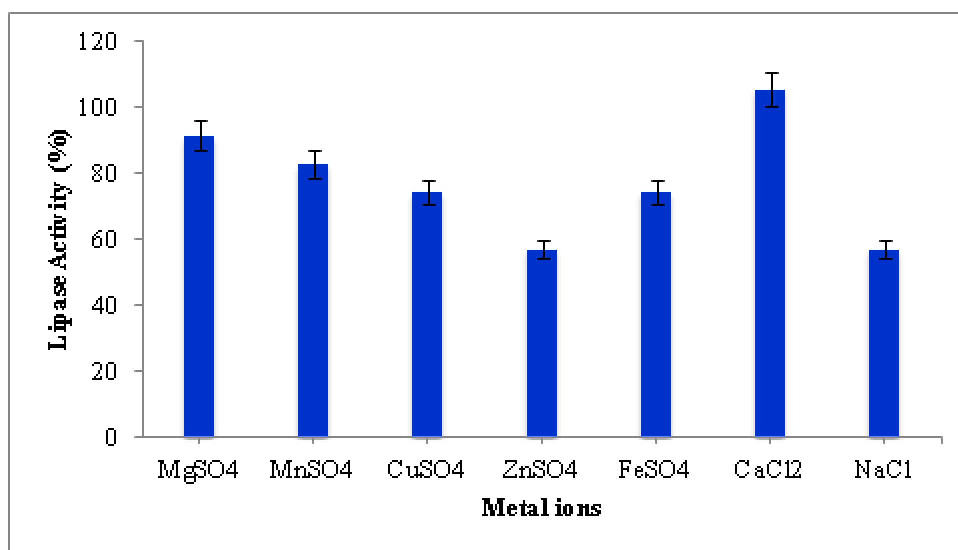

Figure 11. Effect of metal ions on lipase activity. Source: Authors, 2023.

3.3.1.5 Effect of organic solvents on lipase activity

Lipase activity in organic solvents is gaining much industrial importance as the process leads to the development of products of high added value (Verma et al., 2008; Gotor et al., 2002). In our studies, Lipase activity was checked in three different solvents i.e. acetone, methanol and isopropanol with 50% (*v/v*) solution. The enzyme exhibited high activity in all the organic solvents. Maximum lipolytic activity was observed in acetone (106.45%) followed by methanol (101.93%) while isopropanol has no significant effect (100%) (Figure 12).

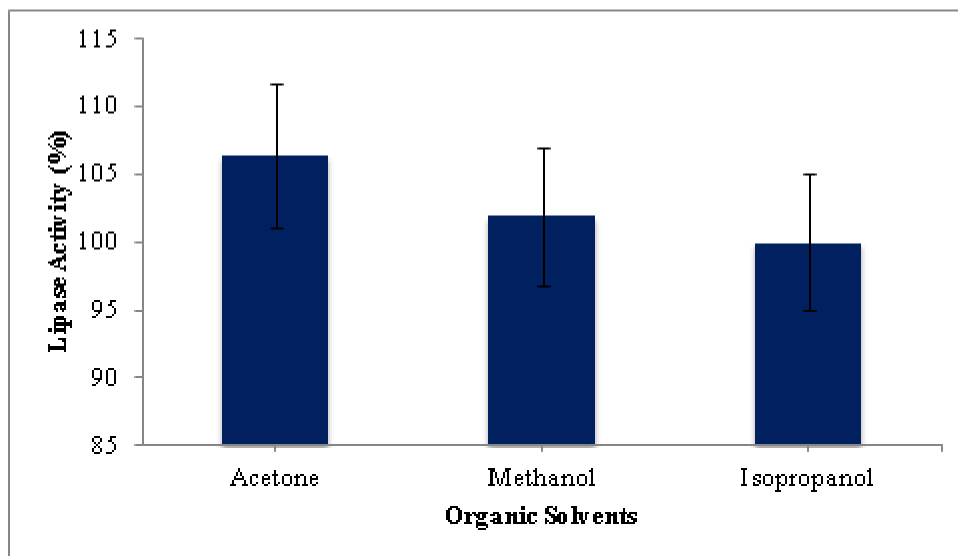

Figure 12. Effect of organic solvents on lipase activity. Source: Authors, 2023.

3.3.1.6 Effect of surfactants on lipase activity

Enzyme to be used in detergent must be stable with all commonly used detergent compounds, in order to be effective during washing purpose (Tiesinga et al., 2007). *Staphylococcus argenteus* MG2 lipase enzyme was found to be appreciably stable in presence of 1% *v/v* of non-ionic detergent like Tween 80 and Triton X-100 when incubated for 2 h at 37 ºC and the % lipase enzyme activities were assayed under standard conditions. Tween 80 slightly inhibited the lipase activity with 90.32% at a concentration of 1% *v/v* while Triton X-100 retained 87.09% of residual activity (Figure 13). Furthermore, lipase enzyme was found to be stable in surfactant (SDS, 1%), retaining approximately 85.16%.

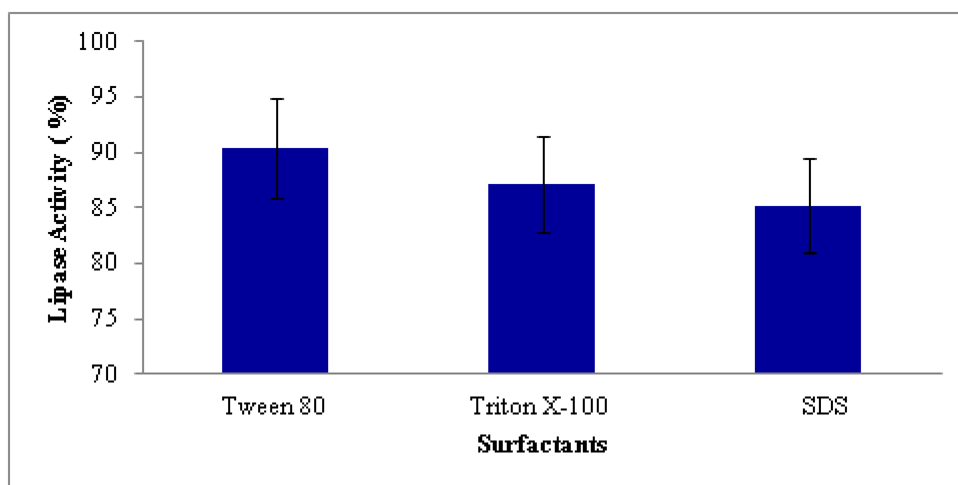

Figure 13. Effect of surfactants on lipase activity. Source: Authors, 2023.

3.3.1.7 Effect of inhibitors on lipase activity

Inhibition studies offer valuable insights into the characteristics of an enzyme, including its cofactor requirements and the nature of its active site (Sigma; Mooser, 1975). Upon examining the influence of various inhibitors on lipase activity, it was observed that the metal chelator EDTA reduced lipase activity to 83.87% at a concentration of 1% *w/v*. Conversely, the reducing agent β-mercaptoethanol decreased lipase activity by 78.09%. Notably, $HgCl_2$ exhibited strong inhibition of the lipase, resulting in residual activity of 70.96% (Figure 14).

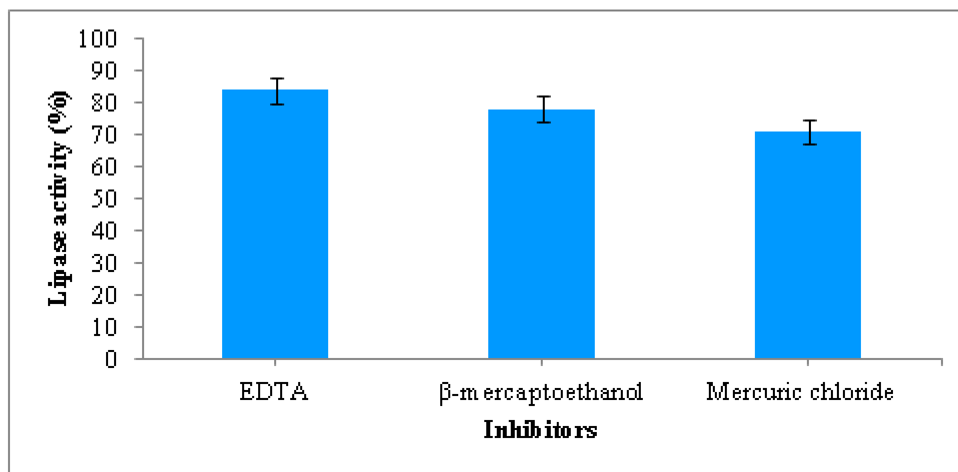

Figure 14. Effect of inhibitors on lipase activity. Source: Authors, 2023.

4. Discussion

The lipase purification process involved sequential steps of ammonium sulphate precipitation, dialysis, ion exchange, and gel filtration chromatography. This process yielded a purification factor of 40.96-fold and a final enzyme recovery of 26.25%, with a specific activity of 744.68 U mg^{-1}. Confirmation of lipase purity was achieved through SDS-PAGE, revealing a single band with a molecular mass of approximately 45 kDa. This finding aligns with previous studies, such as those by Horchani et al. (2009) and Bacha et al. (2016), which reported similar molecular weights for lipases. The molecular weight of the purified lipase of *Staphylococcus epidermidis* strain L2 was found to be 28 KDa and specific activity was 123.95 U mg^{-1} (Sirisha et al., 2017) while the molecular weight of the purified lipase of *Staphylococcus caprae* strain NCU S6 was found to be 87 KDa and specific activity was 502.76 U mg^{-1} (Junxin et al., 2021).

The kinetic parameters of the purified lipase produced by *S. argenteus* MG2 were determined by analyzing the rates of hydrolysis at different concentrations of olive oil. The Lineweaver-Burk plot yielded a Km value of 4.95 mM and a Vmax value of 79.36 μmol/min/mg^{-1}. These values are consistent with previous findings by Horchani et al. (2009), which showed a Km value of 4.93 mM and a Vmax of 77.74 μmol/min/mg^{-1}, further supporting the reliability of our study. The (Km app) 3.22 mM and (Vmax) 50 μmol/min/mg^{-1} of commercial lipoprotein lipase showed the less stability than our studied lipase enzyme.

Each enzyme has an optimum pH for most efficient function. In the present study, the activity profile of the lipase production by *S. argenteus* MG2 at different pH showed that the lipase is stable at a broad range of the pH values between pH 5-12. Our results are in concurrence with other reports in the literature where activity of *Staphylococcus* sp. was found to be stable between pH 5-12 (Horchani et al., 2009, Cherif et al., 2011). The lipase activities were 88.71, 97.14, 100, 94.85, 92.57, 90.71, 88.52, and 87.12% respectively at pHs of 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0. Maximum lipase activity (100%) was obtained at pH 7.0. Alkaline lipase has a great potential for application in the detergent industry (Chen et al., 1998, Illanes et al., 1999).

The enzyme produced by the isolate *S. argenteus* MG2 under study showed good activity in alkaline ranges of pH which indicates that it is suitable for use as an additive in detergents. The lipase activity profile was also studied by performing the enzyme assay at different temperatures between 30-90 ºC. The optimum temperature of lipase obtained from *S. argenteus* MG2 was 30 ºC with 100% lipase activity. The activity remained 88.8% till 90 ºC. This shows it is thermo stable in nature. Our results are in concurrence with studies carried out by Horchani et al. (2009) and Cherif et al. (2011). Thus, alkali stable as well as thermo stable lipase produced by *Staphylococcus argenteus* MG2 can suitably be used in detergent formulations.

Thermo stable and alkaline lipases are highly useful (Illanes et al., 1999) in the synthesis of biopolymers and biodiesel and are used for the production of pharmaceuticals, agrochemicals, cosmetics and flavour (Haki et al., 2003). The effect of metal ions viz. Mg^{2+}, Mn^{2+}, Cu^{2+}, Fe^{2+}, Ca^{2+}, Na^{+}, Zn^{2+} was analysed on the activity of lipase isolated from *Staphylococcus argenteus* MG2. Metal cations, particularly Ca^{2+}, play important roles in influencing the structure and function of enzymes, and calcium ions stimulated lipases have been reported (Haki et al., 2003). It has been demonstrated that the activity of *Staphylococcal* lipases may depend on the presence of

Ca^{2+} ions (Sayari et al., 2001). The effect of various Ca^{2+} concentrations on the rate of hydrolysis of emulsified olive oil by this lipase was studied. Our results showed that lipase activity of this *Staphylococcal* lipase was stimulated by Ca^{2+} to 105.71% at a concentration of 1mM $CaCl_2$ and also showed increase in the thermo stability. The lipase activity of *Staphylococcus xylosus* increased maximally about 3 times at the Ca^{2+} concentration of 10 mM however, 1.9 times increase with 2 mM Ca^{2+} concentration (Mosbah et al., 2005).

Lipase activity in organic solvents is gaining much industrial importance as the process leads to the development of products of high added value (Verma et al., 2008; Gotor et al., 2002). In our studies, lipase activity was checked in three different solvents i.e. acetone, methanol and isopropanol with 50% (*v/v*) solution. In all these organic solvents, the enzyme showed high activity. Maximum lipolytic activity was observed in acetone (106.45%) followed by methanol (101.93%) while isopropanol has no significant effect (100%). The lipase from *Bacillus* sp. was also stimulated in the presence of acetone (Ghori et al., 2011). Stability of lipase enzyme in organic solvents suggests that it is useful in organo synthetic reactions.

Lipase enzyme was found to be stable in surfactant (SDS, 1%), retaining approximately 85.16%. The stability towards SDS is important because SDS stable enzyme have been rarely reported. Cherif et al. (2011) reported 85% of residual lipase enzyme activity in presence of SDS (1%) and the lipase (*Staphylococcus* sp.) was much more stable than in the commercial detergent lipolase (70%). Lipase enzyme was found to be stable in both ionic and non ionic detergents which indicate that it can be used in detergent formulations.

When the influence of various inhibitors on lipase activity was examined, it was observed that the metal chelator EDTA reduced the lipase activity to 83.87% at a concentration of 1% *w/v*. This result suggests that metal ions are essential for the enzyme activity. In few cases EDTA has stimulatory or no effect on lipase activity (Handelsman et al., 1994 and Dharmsthiti et al., 1998), whereas in others it shows inhibitory effect (Talon et al., 1995). Ghori et al. (2011) reported the same result that EDTA and SDS strongly inhibited the lipolytic activity. The reducing agent, β-mercaptoethanol and $HgCl_2$ reduced the lipase activity by 78.09% and 70.96% respectively, indicating that they are reducing the disulphide bonds between cysteine residues of a lipase and prevent the formation of inter or intramolecular disulphide bonds which is essential for enzyme activity.

5. Conclusions

Microbial biotechnology is rapidly advancing, particularly in exploiting microorganisms to produce crucial enzymes. In this study, lipase from the alkali-tolerant *Staphylococcus argenteus* MG2 bacterium was produced, purified, and characterized for its high yield. Its stability across various conditions suggests significant biotechnological potential. Additionally, the immobilized lipase found applications as a detergent additive and in bioremediation efforts targeting lipid-rich wastewater and soil hydrocarbon degradation.

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7. Authors' contributions

Both the authors contributed equally.

8. Conflicts of Interest

No conflict of interest.

9. Ethical approval

Not applicable.

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