CHAPTER 2
Production of Lipase by Staphylococcus arlettae JPBW-1 through SmF and its Characterization
ABSTRACT

Studies on lipase production and characterization were carried out with a bacterial strain Staphylococcus arlettae JPBW-1 isolated from rock salt mine, Darang, India. Higher lipase activity has been obtained using 10 % inoculum with 12 % of soybean oil as a carbon source utilizing a pH 8.0 in 3 h at 35 °C and 100 rpm through submerged fermentation. Partially purified S. arlettae lipase has been found to be active over broad range of temperature (30-90 °C), pH (7.0-12.0) and NaCl concentration (0 to 20 %). It has been shown extreme stability with solvents such as benzene, xylene, n-hexane, methanol, ethanol and toluene up to 30 % (v/v). The lipase activity has been found to be inhibited by metal ions of K⁺, Co²⁺ and Fe²⁺ and stimulated by Mn²⁺, Ca²⁺ and Hg²⁺ metal ions. Lipase activity has been diminished with denaturants, but enhanced effect has been observed with surfactants, such as Tween 80, Tween 40 and chelator EDTA. The \( K_m \) and \( V_{max} \) values were found to be equal to 1.05 mM and 2.61 mmol/min, respectively. Thus, the lipase from S. arlettae may have considerable potential for industrial application from the perspectives of its tolerance towards industrial extreme conditions of pH, temperature, salt and solvent.

2.1 INTRODUCTION

Lipases (triacylglycerol acylhydrolases (E.C.3.1.1.3) are a class of hydrolases that catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil-water interface and can reverse the reaction in aqueous and non-aqueous media (Horchani et al., 2012). Microbial lipases also have been immensely used for biotechnological applications in dairy, detergents, and textile industries, as well as surfactant and oil-processing industries with application versatility from esterified products to pharmaceutical products (Tan et al., 2010). Among different microbial sources, bacterial lipases received much attention for their substrate specificity and their ability to function in extreme environments. Bacterial lipases are mostly inducible enzymes with requirement of triglycerides as inducers (Neihaya et al., 2012). These are mostly extracellular in nature and are produced mainly through submerged fermentation.
Submerged fermentation (SmF) holds tremendous potential for the production of lipases due to usage of this crude one directly as a lipase source for industrial application (Kirk et al., 2002 and Barbosa et al., 2011). Lipase production through SmF is dependent upon a number of factors including carbon and nitrogen sources, pH, temperature, aeration and inoculum size. Optimization of fermentation conditions for microbial lipase is of great interest since culture conditions influence the properties of the enzyme produced as well as the ratio of extracellular and intracellular lipases. Therefore, optimization of the lipase production has been focused on improving fermentation conditions such as carbon or nitrogen source, temperature, pH, aeration, using inducers and source of inoculum, etc. (Gupta et al., 2004 and Sharma et al., 2001). The major requirement for commercial lipases is thermal stability for resisting the chemical modifications caused by high temperatures employed in various industrial lipase catalyzed reactions due to the high melting points of the substrates (lipids). Stability in organic solvents is required since low-water systems based on organic solvents are necessary in order to provide conditions that favour the synthetic reaction over the normal hydrolytic reaction (Ahmed et al., 2009). This has drawn the interest towards thermophiles in both research and industries. Searching for new sources of lipases is justified by realizing variety of future applications requiring not only enzyme–substrate specificity but also process stabilities such as wide pH tolerance and high thermal stability. The stability of these enzymes in organic solvents has pushed them into the frontier areas of organic synthesis leading to the designing of novel drugs, surfactants, bioactive compounds. Industrial demand for the thermostable enzymes continues to stimulate the search of novel thermophilic microorganisms from different unexploited regions of the earth, as small numbers of bacterial strains producing thermophilic lipases have been reported in the last decade (Karatay and Donmez, 2011). Recently, a screening of lipase activity was carried out on halophilic bacteria from salt lake of Yuncheng, China, in this work reported a moderately halophilic strain LY7-8 (Li and Yu, 2012). Recent studies also reported lipolytic enzyme-producing thermophilic microorganism named Bacillus thermoamylovorans, isolated from a hot spring in Galicia (North Western Spain) (Derve et al., 2012).
In the present study, lipase production from *S. arlettae* through SmF has been carried out and partial purification has been made to characterize its stability in the presence of pH, temperature, salt conditions, organic solvents, surfactants, inhibitors and metal ions.

### 2.2 MATERIALS AND METHODS

#### 2.2.1 Chemicals

*p*-nitrophenyl palmitate (Sigma –Aldrich, USA), LB Broth, Miller (Merck, India) were used for the present study. EDTA, o-phenanthroline, PMSF, guanidine thiocyanate, β-6-mercaptoethanol was either of HPLC grade or AR grade was obtained from Merck.

#### 2.2.2 Microorganism and culture maintenance

The bacterial strain, *Staphylococcus arlettae* JPBW-1 used in this study was isolated from the rock salt mine Darang HP, India and identified by 16S rRNA analysis. It was cultured on Luria agar and maintained at 37 °C. The strain was subcultured every two weeks to maintain its viability.

#### 2.2.3 Lipase production through submerged fermentation

Submerged fermentation was carried out by seeding the inoculum size (2 – 12 %) in Erlenmeyer flasks (250 mL) containing 100 mL of the L.B medium (10g of a casein enzymic hydrolysate, 5 g of yeast extract, 10 g sodium chloride per litre) (pH 7 - 12), supplemented with soybean oil (2 – 12 % v/v). Our preliminary results in the selection of 100 ml media has been tabulated under Table 2.1.

**Table 2.1**

<table>
<thead>
<tr>
<th>Vol. of media (ml)</th>
<th>Lipase activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.026 ± 0.60</td>
</tr>
<tr>
<td>50</td>
<td>0.05 ± 0.77</td>
</tr>
<tr>
<td>75</td>
<td>0.1 ± 0.58</td>
</tr>
<tr>
<td>100</td>
<td>0.19 ± 0.46</td>
</tr>
<tr>
<td>125</td>
<td>0.08 ± 0.84</td>
</tr>
<tr>
<td>150</td>
<td>0.035 ± 0.62</td>
</tr>
</tbody>
</table>

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The effects of different carbon sources (coconut oil, olive oil, and soybean oil) were estimated in relation to enzyme activity. Lipases are inducible enzymes, generally produced in presence of lipids. In the present study soybean oil (lipid source) acts as an inducer for lipase production.

2.2.4 Lipase assay and protein estimation

Lipase activity was assayed quantitatively using p-nitrophenyl palmitate as the substrate according to the method described by Kordel et al., 1991. One enzyme unit was defined as the amount of enzyme that liberates 1 μmol of 4-nitrophenol per minute under the assay conditions. Protein content of cell-free supernatant was determined according to modified Lowry method (Lowry et al., 1951) using bovine serum albumin as standard.

2.2.5 Partial purification of lipase

To precipitate lipase by ammonium sulphate experiment was conducted at 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 % and 90 % saturation of ammonium sulphate salt under stirring conditions and maintained for 60 min at 4 °C. The precipitate was collected by centrifugation at 6314 g for 15 min and was dissolved in a minimum quantity of 50 mM Tris-HCl buffer (pH 8) and precipitated protein was dialyzed against buffer for 24 hr. Then the lipase assay and protein estimation was performed.

2.2.6 Characterization of lipase

The pH stability of lipase was tested by incubating the enzyme at different pH ranging from 4 to 12 using different buffers (0.5 M; acetate, phosphate, Tris-HCl and glycine and NaOH,) at 35 °C for 1 h, following by standard enzyme assay and reported in terms of relative activity. The effect of temperature (30 – 90 °C) on alkaline lipase activity was determined by incubating the reaction mixture for 1 h and relative activity was calculated. To study the effect of NaCl concentration, aliquots of the enzyme were incubated with different concentrations of NaCl (0 – 25 %) for 60 min at 35 °C and relative activity was measured under standard assay conditions. The effect of organic solvents on lipase was determined by incubating enzyme solution in different organic solvents at 35 °C, for 1 hr. Effect of different surfactants on the lipase activity was investigated by pre-incubating the enzyme for 60 min at 35 °C in Tris-HCl buffer (50 mM, pH 8.0) containing 1 mM Tween 40, Tween 20 and Triton-X 100. To determine
the effect of different chloride salts of the metal ions (1 mM; Co²⁺, Ca²⁺, K⁺, Mg²⁺, Hg²⁺, Fe²⁺, Mn²⁺ and Na⁺) on lipase activity was investigated by pre-incubating the enzyme with metal ion (1:1 ratio) for 1 h at 35 °C and then, relativity activity was determined. The effect of chelators (EDTA, α-phenanthroline) and denaturants (PMSF, guanidine thiocyanate, β-6-mercaptoethanol) on the lipase activity were examined at concentration of 1 mM at 1:1 ratio for 1 h at 35 °C, and relativity activity was determined. In all these cases, the control used was the untreated enzyme under the same experimental conditions and relative activity was calculated taking the value of control as 100 %. The Michaelis-Menten constant (Kₘ) and the maximum velocity for the reaction (Vₘₐₓ) were determined from Lineweaver-Burk plot.

2.3 RESULTS AND DISCUSSION

2.3.1 Selection of parameters for lipase production through SmF

2.3.1.1 Selection of incubation temperature

For selection of optimum temperature for the production of lipase, the temperature was varied from 25 °C to 50 °C, keeping the other process conditions same. It was seen that the maximal lipase activity (0.216 U/ml) has been seen at 35 °C (Fig.2.1).

![Figure 2.1: Selection of incubation temperature for lipase production. All values are represented as ± s.d of three replications. (Experimental conditions: Inoculum size- 5 %, Incubation period - 2 h, Agitation speed-100 rpm, pH- 7).](image)

Mohan et al., 2008 found that an optimum temperature for lipase production by Bacillus sp. was 37 °C in his study that coincides with this study. Similarly, Walavalkar and
Bapal, 2002 have reported that, the lipase activity of *Staphylococcus* sp. was high at 37 °C. Thermophilic lipases have a high potential in the detergent and food industries and therefore, the organism may be exploited and scaling up could be attempted for industrial production.

### 2.3.1.2 Selection of inoculum size

The effect of inoculum size on lipase activity was determined at different inoculum size ranging from 2% to 14% at 35 °C. A maximum lipase activity of 0.349 U/ml has been observed using 10% of inoculum. The cultures were incubated for 48 h and the lipase production was studied. After a certain concentration, the lipase activity increased and then decreased (Fig. 2.2). This may be due to overpopulated culture and fixed amount of nutrient with which the organism starts liberating proteolytic enzyme, enhancing self consumption (Basheer et al., 2011).

![Figure 2.2: Selection of inoculum size for lipase production. All values are represented as ± s.d of three replications. (Experimental conditions: Incubation period- 2 h, Temperature - 35 °C, Agitation speed 100 rpm, pH - 7)](image)

### 2.3.1.3 Selection of media pH

The pH of the production medium plays a critical role for the optimal physiological performances of the bacterial cell and the transport of various nutrient components across the cell membrane aiming at maximizing the enzyme yields. *Staphylococcus* sp. was inoculated in the lipase production medium and incubated at different pH (4-11). At pH 9, maximum lipase activity was observed (0.539 U/ml) (Fig. 2.3). A comprehensive
review of all bacterial lipase done by Gupta et al., 2004 states that maximum activity of lipases at pH values higher than 7 has been seen in many cases. Generally, bacterial lipases have neutral (Dharmshiti and Luchai, 1999 and Lee et al., 1999) or alkaline pH optima (Salihu et al., 2011; Kanwar et al., 2002; Sunna et al., 2002) with the exception of *P. fluorescens* SIK W1 lipase, which has an acidic optimum at pH 4.8 (Andersson et al., 1979).

![Graph showing pH and Lipase Activity](image)

**Figure 2.3:** Selection of pH of media for lipase production. All values are represented as ± s.d of three replications. (Experimental conditions: Inoculum size – 10 %, Incubation period – 2 h, Temperature – 35 °C, Agitation speed- 100rpm)

### 2.3.1.4 Selection of incubation time

The effect of incubation time on lipase activity was determined for 1 – 15 hrs. It was noted that high lipase activity (0.628 U/ml) has been found with incubation time of 3 h (Fig.2.4). At longer incubation periods, the lipase activity decreased which might be due to the depletion of nutrients, accumulation of toxic end products, and the change in pH of the medium, or loss of moisture. Several researchers have reported different incubation periods for optimal lipase production. Incubation periods ranging from few hours to several days have been found to be best suited for maximum lipase production by bacteria. The incubation period of 12 h was optimum for lipase production by *A. Bacillus sp.* RSJ1 (Sharma et al., 2002) and 16 h for *B. thermocatenulatus* (Schmidt et al., 1997).
Figure 2.4: Selection of Incubation time for lipase production. All values are represented as ± s.d of three replications. (Experimental conditions: Inoculum size – 10 %, Temperature -35 °C, Agitation speed -100 rpm, pH-8.0)

2.3.1.5 Selection of carbon source

The culture environment has a dramatic influence on enzyme production especially carbon and nitrogen sources play a crucial role in enzyme induction, in bacteria. A major factor for the expression of lipase activity has always been carbon, since lipases are inducible enzymes and are thus produced in the presence of a lipid source such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts and glycerol.

Figure 2.5: Selection of carbon source (a) and its % (b) for lipase production. All values are represented as ± s.d of three replications. (Experimental conditions: inoculum size -10 %, incubation period -5 h, Temperature – 35 °C, pH- 8.0, Agitation speed- 100rpm)
The increasing concentration of soybean oil increased the lipase activity (till 1.263 U/ml), but it has also been observed that, after a certain limit, the lipase activity fell down sharply (Fig 2.5). This may be attributed to the substrate inhibition (Rathi et al., 2001; Alfrod and Smith, 1965).

2.3.1.6 Selection of agitation speed

To evaluate the effect of agitation speed on lipase production by *S. arlettae*, experiments were carried out at different agitation speeds ranging from 50 to 200 rpm at 35 °C. It has been seen that, in the stationary condition, there was no lipase secretion, whereas, in shaking condition, a considerable amount of lipase activity was obtained when all other conditions were same. It was observed that maximum lipase activity has been observed (2.162 U/ml) utilizing an agitation speed of 100 rpm (Fig 2.6). From the results, the authors can interpret that the micro-organism responsible for lipase production had very strong affinity for oxygen for its metabolic activity particularly for the synthesis of lipolytic enzyme. This is not the first report which gave evidence of the negative effect of the higher mixing rates on the lipase production by *Geotrichum candidum*.

![Figure 2.6: Selection of agitation speed for lipase production. All values are represented as ± s.d of three replications. (Experimental conditions: Inoculum size- 10 %, Incubation period -3 h, Temperature – 35 °C, pH-8.0, Carbon source- 12 % soybean oil)](image)

Alford and Smith, 1965 reported that the lipase yields reduced for 60 % as a result of the mixing at low rates and even more at the higher ones. Wouters, 1987 similarly to the
previous case, reported that the growth and the lipase production by *Geotrichum candidum* decreased as the aeration or agitation rate of the culture medium increased.

**2.3.1.7 Partial purification of lipase by ammonium sulphate precipitation**

The ammonium sulphate precipitation was carried out by addition of varying concentration (10 % to 100 %) of ammonium sulphate under stirring conditions. The precipitated protein was collected through centrifugation (6987 g, 10 min) and dissolved in 50 mM Tris-HCl (pH 8.0) and assayed for lipase activity. It was found that at 60 % ammonium sulphate concentration, the specific activity of lipase was more. A purification fold of 3.72 and enzyme yield of 31 % was obtained. Chahinian et al., 2000 and Hiol et al., 2000 were reported the effective recovery of extracellular lipases from *Penicillium cyclopium* and *Rhizopus oryzae* respectively through ammonium sulphate precipitation. Salting out through ammonium sulphate stabilizes the proteins against denaturation, proteolysis and bacterial contamination (Roe, 2001).

**2.3.2 Characterization of lipase**

**2.3.2.1 Effect of pH on lipase activity**

Changes in pH will affect the protein structure and the enzyme activities (Ohnishi et al., 1994). The effect of pH on lipase activity is shown in Fig. 7, where lipase showed activity in the pH range of 7.0-12.0. The activity was highest at pH 9 (0.5 M Tris buffer). However lipase retained 99 % and 102 %, of relative activity for 24 h at pH 8, and 9, respectively and very less activity has been observed at pH 4 and pH 5.0 respectively. So the enzyme was only active in alkaline pH range. Kumar et al., 2005 reported that the purified lipase from *B. coagulans* BTS-3 was stable within a pH range of 8.0–10.5 with optimum activity at pH 8.5. Stability of the enzyme at different pH values was determined by measuring the residual activity after incubating the enzyme in the pH range of 4–12 for 1 h at 4 °C. The enzyme exhibited maximum stability at pH 9 and comparable stability also observed at pH 12 (91 %). Fig 2.7 depicts that the enzyme showed stability in the pH range of 7 to 12.
2.3.2. Effect of temperature on lipase activity

The effect of temperature on lipase activity was analyzed by carrying out assays at different temperatures (30 °C – 100 °C). With the rise in temperature, the enzyme activity started increasing and reached an optimum at 37 °C (Fig. 2.8). Our newly isolated *Staphylococcus* sp. lipase was stable against thermal denaturation where it remained 62 % of its original activity at 90 °C after 60 min. Since thermostable lipases, which are active and stable in acidic and alkaline media, are very attractive and have a high potential for different industrial applications, this salt mine isolated *Staphylococcus* sp. lipase would be a potent and valuable enzyme for further applications. It has been reported that the drop in the percentage of residual activity at high temperatures results first in some conformational changes in the tertiary structure, and then almost complete inactivation of the enzymes (Ozen et al., 2004). In contrast to *Staphylococcus similans* lipase which is inactivated after a few minutes when incubated at 60°C (Sayari et al., 2001) *Sarlattae* retained 90 % or 60 % of its activity up to 60 min at 55 – 60 °C, respectively.
Figure 2.8: Effect of temperature on lipase activity. All values are represented as ± s.d of three replications

2.3.2.3 Effect of NaCl concentration on lipase activity

The effect of NaCl concentration lipase activity was studied by incubating the enzyme with different percentage of 0 to 30 % of NaCl. From Figure 2.9, it was observed that S. arlettiae lipase could produce in 0- 25 % NaCl but the best growth was seen in medium without NaCl.

Figure 2.9: Effect of different NaCl concentration on lipase activity. All values are represented as ± s.d of three replications
This showed that *Staphylococcus arlettae* should be classified as halo-tolerant bacteria. Enzyme stability at high salt concentrations might indicate that the enzyme will be stable in the low water activity environments that occur in biocatalytic reactions carried out in organic solvents (Eichler, 2001).

### 2.3.2.4 Effect of organic solvents

Exposure of the *S. arlettae* to various organic solvents for 60 min showed that this enzyme retained activity in all organic solvent tested. The highest relative activity was achieved at 168.0 %, 143.0 %, and 141.8 % in xylene, benzene, and toluene, respectively (Fig 2.10). However, the activities were decreased when the enzyme incubation were extended to 2 h in organic solvents. Staphylococcus lipase was very stable when incubated with benzene, xylene, n-hexane, methanol, ethanol and toluene below 30 % (v/v) but was stability reduced drastically above 40 %. The stability of *S. arlettae* in aqueous-organic mixtures suggested the ability of this enzyme to retain activity in organic solvents and held the potential for its use in organic synthesis and related applications. High activity and stability of lipases in organic solvents is an essential prerequisite for applications in organic synthesis (Doukyu and Ogino, 2010; Ogino and Ishikawa, 2001). Hence stability in organic solvents are considered unique attributes in a lipase.

![Figure 2.10: Effect of organic solvents on lipase activity. All values are represented as ± s.d of three replications](image)

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2.3.2.5 Effect of metal ions on lipase activity

The effect of different metal ions were tested on lipase activity at 1 mM concentration in 50 mM Tris HCl buffer (pH 8.0) for 1 hr. Previously, it has been demonstrated that the activity of Staphylococcal lipases may depend on the presence of Ca\(^{2+}\) ions (Sayari, 2001). It has been reported that the lipases from P. glumae (El et al., 2003) and S. hyicus (Rosenstein and Gotz, 2000) contain a Ca\(^{2+}\)-binding site which is formed by two conserved aspartic acid residues near the active-site, and that binding of the Ca\(^{2+}\) ion to this site dramatically enhanced the activities of these enzymes. Kambourova et al., 2003 suggested that the positive effect of Ca\(^{2+}\) is due to the formation of insoluble ion-salts of fatty acids during hydrolysis, thus avoiding the product inhibition. It was observed that, in the presence of 1 mM CaCl\(_2\) and MnCl\(_2\), the enzyme activity increased. All the other metal ions inhibited the activity of lipase (Fig. 2.11).

![Figure 2.11](image)

**Figure 2.11** Effect of different metal ions on lipase activity. All values are represented as ± s.d of three replications

2.3.2.6 Effect of surfactants and vitamins on lipase activity

Surfactants usually increase the permeability of the cell wall. The higher concentration of surfactants may have adverse effects on the physiology of the organism causing lower yield due to partial denaturation of the enzyme. All the surfactants used in the present study had an inducing effect on lipase production. The effect was the maximum in presence of Tween 40 (Fig. 2.12). Stimulating effect of surfactants on enzymatic
hydrolysis has been reported many times (Kristensen et al., 2007). Ebrahimpour et al., 2011 and Castro-Ochoa et al., 2005 found that lipase activity of Bacillus sp. was enhanced in the presence of Triton X-100. The Lip-SBRN2 exhibited a high level of activity in the presence of SDS (Kankanavas et al., 2010). It was observed that lipase production was affected in the presence of vitamins. Vitamins act as prosthetic groups for many enzymes. The lipase activity was found to be a maximum in the presence of nicotinic acid (Fig.2.13) Nicotinic acid stimulated maximum lipase in Curvularia pellessens, Fusarium equiseti and Trichoderma viridae (Kakde and Chavan, 2011).

![Figure 2.12](image)

**Figure 2.12** Effect of surfactants on lipase activity. All values are represented as ± s.d of three replications

2.3.2.7 *Effect of inhibitors and chelators on lipase activity*

The lipase of S. arleatte when incubated with 1 mM serine protease inhibitors, PMSF β-6-mercaptoethanol and guanidine thiocyanate for 1h showed drastic inhibition in lipase activity. Inhibition of activity with serine inhibitors shows that this lipase belongs to the class of serine hydrolases (Yadav et al., 1998 and Gilbert et al., 1991). The lack of sulphur containing amino acid in lipase active site has been confirmed through the lipase activity inhibition with β-mercapto ethanol and guanidine thiocyanate (Brzozowski et al., 1991). The chelators, EDTA and o-phenanthroline were studied for their influence on lipase activity at a concentration of 1 mM. The enzyme activity has been seen to increase in presence of 1 mM EDTA and lipase activity has been diminished with 1 mM o-phenanthroline (Fig. 2.14). Enhanced and diminished activities...
in the presence of EDTA and o-phenanthroline has attributed to the non-requirement and requirement of cofactor for lipase activity respectively (Yadav et al., 1998 and Brzozowski et al., 1991).

![Graph showing the effect of vitamins on lipase activity](image1)

**Figure 2.13** Effect of vitamins on lipase activity. All values are represented as ± s.d of three replications.

The inhibition results with o-phenanthroline have been also reported in case of *Penicillium chrysogenum* (Bancerz et al., 2005).

![Graph showing the effect of chelators on lipase activity](image2)

**Figure 2.14** Effect of chelators on lipase activity. All values are represented as ± s.d of three replications.

### 2.3.2.8 Kinetic constants of *S. arleatte* lipase

Basic enzyme kinetics such as $K_{m}$ and $V_{max}$ are adopted to describe the dynamic behavior. The kinetic constants $K_{m}$ and $V_{max}$ have been seen to be equal to 7.05 mM and 2.67 μmol/min, respectively for *S. arleatte* lipase using p-NPP as substrate at 35 °C, pH 8.0 through Lineweaver –Burk plot (Fig. 2.15).
The high affinity of the enzyme for p-NPP is reflected in the relatively low \( K_m \) value. Similar results have been reported in case of lipase from *Staphylococcus aureus* and *P. cepacia* using p-NPP as substrate. The kinetic constants of lipase from *Staphylococcus aureus* have been reported as 14.53 mM and 1485 \( \mu \text{M/min/mg} \) for \( K_m \) and \( V_{\text{max}} \) values, respectively (Horchan et al., 2009). Pencrea'c'h and Baratti (1996) reported \( K_m \) and \( V_{\text{max}} \) values of 12 mM and 30 mmol/min, respectively for *P. cepacia* lipase using p-NPP as a substrate.

![Graph](image_url)

**Figure 2.15. The Lineweaver-Burk plot of *S. arletta* lipase**

### 2.4 CONCLUSIONS

The properties of a novel, halo - thermo - solvent- detergent tolerant lipase by *Staphylococcus arlettae* showed many advantageous features for industrial applications, which may be helpful for possible application in the detergent industry, leather, pharmaceutical, cosmetic industry. This lipase was characteristically stable at 30–90 °C, pH 7.0–12 and 0 -20 % NaCl. Enzyme activity was stimulated by Ca\(^{2+}\), Hg\(^{2+}\) and Mn\(^{2+}\), and inhibited by K\(^+\), Zn\(^{2+}\), and Co\(^{2+}\). Additionally, the enzyme was strongly inhibited by PMSF, β-mercaptoethanol but not affected by EDTA. The PMSF inhibition showed that the key enzyme was a serine hydrolase. All of these results led us to conclude that the enzyme may have considerable potential for industrial application from the perspectives of its properties.