Thermostable, alkaline and detergent-tolerant lipase from a newly isolated thermophilic *Bacillus stearothermophilus*

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Lipases (glycerol ester hydrolases, EC 3.1.1.3) catalyze the hydrolysis of triglycerides and several other substrates containing ester bonds\(^1,2\) with an astonishing variety in their regio- and stereo- specificity, as well in their selectivity towards the length and saturation degree of the acyl chain. They represent an important group of biotechnologically valuable enzymes\(^3\). They are widely spread in nature and are isolated from different sources, such as plants, animals and microorganisms. Microbial lipases extracted from thermophilic microorganisms have received much attention during the past decades, since they have a great potential in terms of lower energy costs and microbial contamination in industrial processes\(^4,5\) and high specificity of reaction both hydrolysis and synthesis, enabling the use of hydrophobic substrates\(^6\) and improving thermal stability of the enzyme\(^7\). Lipases are also important drug targets or marker enzymes in the medical sector. They can be used as diagnostic tools and their increasing levels can indicate some kinds of infections (such as gallbladder infection) or diseases\(^8\).

Bacterial lipases have received much more attention due to their substrate specificity and ability to function in extreme environments (i.e. extreme temperature, pH, salts concentrations and in the presence of organic solvents). They are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors, such as temperature, pH, nitrogen and carbon substrates, inorganic salts, agitation, dissolved oxygen concentration and presence of inducers\(^9,10\).

As each industrial application requires specific lipase, there is still an interest in additional lipases that could be used in new production processes\(^11\). Therefore, screening of new microorganisms with lipolytic activities could facilitate the discovery of novel lipases.

Oil mill soil is one of the potential sites which may contain lipolytic bacteria. The oily environment may provide a good source for lipolytic microorganisms to flourish. The enzyme formation is largely dependent on the condition of growth of the culture and composition of nutrient medium. Here, we report the purification

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and characterization of a novel alkaline thermoactive lipase (BL1) from an alkaline lipase producing strain, identified as Bacillus stearothermophilus collected from olive oil mill soils. Its compatibility with various surfactants, oxidizing agents, organic solvent and commercial detergents has been evaluated for its potential for detergent formulation.

Materials and Methods
Sample collection and screening of lipase producing bacteria
Soil sample contaminated with olive oil was collected in sterile bags and stored in the laboratory at -20°C. 1 g of soil samples were serially diluted for initial screening of lipolytic microorganisms using a plate assay on triacylglycerol and the fluorescent dye rhodamine B medium. The solid medium contained 1% olive oil, 1% nutrient broth, 1% NaCl, 1.5 g agar and 1% rhodamine B. The culture plates were incubated at 37°C and colonies giving orange fluorescence halos around them upon UV irradiation were selected, isolated and purified on nutrient agar N.A (Oxoid) medium for further identification analysis.

Ability of selected strains to produce lipase was also tested in tributyrin (TC4) agar medium containing TC4 oil 1% (w/v). The pH was maintained at 7. The lipolytic activity was determined by measuring the diameter of hydrolytic zones around each colony. The isolate with higher lipolytic activity was used for further process. The identification of this strain with the determination of its morphological aspect and biochemical properties using API 50 CH showed 99.7% identity with Bacillus stearothermophilus.

Lipase production medium
Media used for lipase production contained (% w/v): Glucose 0.1, yeast extract 0.02, CaCl2 0.01, K3HPO4 0.05 (pH 7.0). Glucose was sterilized separately and aseptically added to the flasks containing the liquid medium after cooling. The pre-culture was cultivated in nutrient broth medium (0.8% w/v) for 18 h. Then, overnight cultures were inoculated at 1% in enzyme production media and incubated at 37°C for 80 h in a shaking incubator (150 rpm). Samples were collected every 4 h and centrifuged at 12,000 rpm for 10 min The supernatant was considered as crude enzymatic extract and used for enzyme assay.

Lipase activity determination
The lipase activity was measured titrimetrically at pH 11 and 55°C with a pH-stat under standard conditions using TC4 (0.25 mL) in 30 ml of 2.5 mM Tris–HCl pH 11, 1 mM CaCl2, 1 mM sodium cholate (NaDC) or olive oil emulsion (10 mL) in 20 mL of 9% NaCl pH 11, 1 mM CaCl2, 1 mM NaDC) as substrate. The olive oil emulsion was obtained by mixing 10 ml of olive oil in 90 ml of 10% (w/w) gum arabic (for 3 x 30 s in a Waring blender). Lipolytic activity was expressed in international units. One international unit (1 U) corresponds to 1 µmol of fatty acid released per min.

Effect of incubation time and agitation speed
The effect of incubation time on cell growth and lipase production was determined by incubating the production medium and experimenting the enzyme activity at 37°C and at different time intervals (0–80 h) with an interval of 4 h. Bacterial biomass was determined by measuring the absorbance at 600 nm.

To evaluate the impact of agitation speed on lipase production by Bacillus sp., experiments were carried out at different agitation speeds ranging from 150-230 rpm at 37°C and pH 7. With 1% (v/v) of inoculum, cultures were incubated for 48 h and the lipase production was studied.

Effect of temperature and pH
The pH effect was studied by adjusting the initial pH of the lipase producing medium to a broad pH range from 4 to 13 and then inoculating with the lipolytic strain in study after incubation at 37°C for 48 h. For temperature optimization process, Bacillus sp. was inoculated in lipase production medium at different temperatures ranging from 20 to 70°C. Growth was followed by measuring the cultures OD at 600 nm and lipase activity was determined by assay method. The experiments were conducted in duplicate and the results were the mean of these two trials.

Effect of nutrients and inducers on the enzyme production
The effects of some nutrients, such as carbon and nitrogen sources, some metal salts and inducers, such as triglycerides and detergents on the enzyme production were investigated. Glucose (1% w/v) was replaced in the production medium with lactose, galactose, sucrose, starch, xylose, sorbitol and mannitol. Different organic and inorganic nitrogen sources, including peptone, tryptone, gelatin, skimmed milk powder, soya meal, ammonium chloride, ammonium nitrate and ammonium dihydrogen phosphate were tested. These nitrogen sources were used to replace yeast extract which was the original
nitrogen source in the growth medium. The culture medium was supplemented with the following metal salts (0.1% w/v): zinc chloride, magnesium chloride, barium chloride, mercuric chloride, cobalt chloride, magnesium sulphate, zinc sulphate, copper sulphate and ferric chloride. Incubation period was 48 h at 55°C.

The inducing effect was also studied by adding 1% of several oils and surfactants, namely olive, nut, sesame, soybean and sunflower oils, and TC₄, CHAPS, PEG 200, SDS, Tween 20, Tween 80 and triton X-100 to the flasks at the beginning of the cultures and incubated for 48 h at 55°C. The experiments were conducted in duplicate and the results were the mean of three trials.

A new medium, including the best source of each of carbon, nitrogen, detergent, triglycerides and metal ions for lipase production was improved and bacteria were grown in this new medium.

**Purification of lipase**

The supernatant containing extracellular lipase produced in the optimized medium with the best carbon and nitrogen sources was incubated for 5 min at 70°C. After rapid cooling, insoluble material was removed by centrifugation for 30 min at 12,000 rpm. Thereafter, the pH of the previous supernatant was brought to 7 using 6 N NaOH and centrifuged for 20 min at 12,000 rpm to obtain a clear supernatant containing approximately 81% of the starting lipase activity. The resulting sample was then dialyzed overnight against buffer A (50 mM Tris–HCl buffer, pH 8, containing 0.1 M NaCl and 0.05% Triton X-100) at 4°C. The lipase was then purified to apparent homogeneity by a single CM-Sephadex chromatography step. The dialyzed sample was chromatographed on a 2 cm × 20 cm column of CM-Sephadex, equilibrated with buffer A. Non-bound proteins were removed by rinsing the column with 0.2 M NaCl of buffer B. The adsorbed proteins were eluted with a linear gradient of NaCl (0.2 to 0.6 M). Fractions of 2.0 ml were collected every 6 min and assayed for protein and lipase activity. Fractions with high lipase activity were pooled and concentrated. The samples were then stored at 4°C until used.

**Protein analysis**

Protein concentration was measured spectrophotometrically according to the Bradford method¹⁷, using bovine serum albumin as a standard. Twenty micrograms of the purified lipase was subjected to SDS-PAGE¹⁸ and electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane. The blotted PVDF membrane was stained with 0.1% Coomassie brilliant blue R-250 containing 1% acetic acid and 40% methanol. After washing with 50% methanol, the region containing the lipase band was cut-off from the membrane. The N-terminal amino acid sequence of the protein on PVDF membrane was determined by the Edman’s degradation method on an Applied Biosystems Protein Sequencer Procise 492, equipped with 140 C HPLC systems¹⁹.

**Lipase characterization**

**Kinetic study**

Lipase activities were measured as a function of various substrates, such as TC₄, trictanoin (TC₃) or triolein (TC₁₈) concentrations (0-40 mM). The Michaelis-Menten constant (Kₘₐₓ) and maximum velocity (Vₘₐₓ) for the reaction with TC₄, TC₃ or TC₁₈ as substrates were calculated by Lineweaver-Burk plot.

**Effect of pH and temperature on BL1 activity and stability**

Lipase activity was tested in various buffers at different pH (7–13) at 55°C. The pH stability of the lipase was determined by incubating the enzyme for 24 h at room temperature and at various pH values ranging from 3 to 13 using the following buffers: 200 mM sodium acetate buffer (pH 3-5), 200 mM potassium phosphate buffer (pH 6-7), 200 mM tris-HCl buffer (pH 8-9) and 200 mM glycine-NaOH buffer (pH 10-13). During incubation period, the residual lipase activity was determined after centrifugation at different times and under standard assay method. Each measurement was performed three-times.

The optimum temperature for the purified lipase activity was determined by carrying out the enzyme assay at different temperatures (30-75°C) at pH 11. The thermal stability was studied by incubating the enzyme at pH 11 and at different temperatures (30-75°C) and measuring the residual activity with time, after centrifugation under standard titrimetric assay conditions.

**Compatibility of BL1 with surfactants, oxidizing agents and commercial detergents**

BL1 was characterized for its potential application in the detergent industry. The lipase sample was incubated in the presence of surfactants, oxidants and different kinds of commercial detergents at 1% for 1 h
at 40°C. The endogenous lipases present in these detergents were inactivated by heating the detergents for 1 h at 65°C prior to the addition of enzyme preparation and the residual lipase activity was determined under standard titrimetric assay conditions. The relative activity (%) of each sample was determined and compared with the control without detergent. The relative activity of control was defined as the enzyme activity without surfactant, detergent or oxidizing agent, incubated under similar conditions and was taken as 100%. All the experiments of characterization study were performed three times.

**Effect of organic solvents on BL1 stability**

Pure lipase samples (1 mg/ml) were incubated in each organic solvent (50%) for 1 and 2 h at 25°C and 200 rpm. The residual enzyme activity relative to the non-solvent containing control was measured on olive oil emulsion substrate. Values were average of assays carried out in triplicate.

**Results and Discussion**

**Isolation and characterization of lipolytic strain**

Recently, the emphasis has been laid on extreme thermophiles for their potential use in the production of active and stable thermoenzymes. Most commercial lipases are produced from microbes and the discovery of these thermostable enzymes decreases the cost and increased efficiency. In the present study, from olive oil mill soil samples, more than 37 different isolates were obtained, but only one strain showed maximum orange fluorescence halo when plated on rhodamine B agar base, indicating the presence of a lipase. The lipolytic microbe was identified as Gram-positive, rod-shaped motile organism (Table 1). In addition, biochemical tests using API 50 CH galleries indicated that the organism showed 99.7% identity to *Bacillus stearothermophilus*.

**Optimization of medium components and conditions for lipase production**

In order to improve growth and/or favor the enzyme secretion, the influence of some key variables such as medium composition (i.e., nitrogen, carbon, metal ion, lipid compounds and surfactants) and culture conditions (i.e., pH, temperature, incubation time and agitation speed) was assessed.

**Effect of incubation time on lipase production**

Figure 1A shows the time-course of lipase production followed at 37°C with cell growth. The lipase activity was observed to start soon after incubation and reached the maximum (146 U/ml) at the end of exponential phase, corresponding to 48 h of incubation.

![Fig. 1—(A) Time-courses cell growth of *Bacillus stearothermophilus* and extracellular lipase production (The culture was carried out at 37°C in shaking at 200 rpm. Cell growth was monitored by measuring the absorbance at 600 nm; and (B) Effect of different agitation speeds on extracellular lipase production by *B. stearothermophilus* strain)](image-url)
cultivation. After 48 h of incubation, \textit{B. stearothermophilus} entered the stationary phase and a decrease in enzyme activity, probably due to the presence of proteases in the culture medium, was observed (Fig. 1A). Previous studies have shown maximum lipase production by \textit{Bacillus} sp. between 15–24 h\textsuperscript{22-24}. Thus, this interesting property could allow harvesting of the enzyme for the shorter period of time.

**Effect of agitation speed, pH and temperature on lipase activity**

Assay of the lipase activity at different agitation speeds within the range of 150-230 rpm indicated 200 as the optimum agitation speed for maximum lipase production (Fig. 1B). Furthermore, pH and temperature are the two important environmental factors which influence lipase production. The pH of production medium plays a critical role for the optimal physiological performance of the bacterial cell and transport of various nutrient components across the cell membrane aiming at maximizing the enzyme yields\textsuperscript{9,10}.

The effect of pH on the enzyme production assessed in a wide pH range (4.0–13.0) showed the maximum values of lipolytic activity (325 ± 41.14 U/ml) obtained after 48 h of growth (Fig. 2A). These results suggested that the microorganism needed neutral or alkaline pH values between 7.0 and 13.0. For pH values beyond this range, cell growth seemed to be completely inhibited. This result was in consistence with the earlier studies demonstrating optimal production of lipase by \textit{Bacillus megaterium} AKG-1\textsuperscript{25} and \textit{Bacillus stearothermophilus} MTCC 37\textsuperscript{24} at pH 7.5. A similar trend has been observed in \textit{Bacillus thermocatenulatus}\textsuperscript{26} and \textit{Bacillus cereus} MSU AS\textsuperscript{27} at pH 7.4 and 8, respectively. The use of lipase that is active at relatively alkaline pH is of great industrial application, especially in detergent industries in removing the dirt\textsuperscript{28-29}.

Likewise, temperature may affect the lipase production\textsuperscript{30}. In the present study, the enzyme showed a trend towards thermostolerance; the optimum temperature for the maximum lipase production was 55°C (700 ± 28.28 U/ml) after 48 h of incubation and at 70°C, the enzyme quantity was good (428 ± 35.35 U/ml) (Fig. 2B). Results with \textit{Bacillus stearothermophilus} MTCC 37\textsuperscript{24} and \textit{Bacillus cereus} MSU AS\textsuperscript{27} strains have shown the maximum lipolytic activity when incubated at 50°C. Also, the highest amount of lipase production (49.5 ± 1.7) U/mL by \textit{Bacillus} sp. is found to be at 50°C through submerged fermentation\textsuperscript{31}. However, other studies have reported that the optimum temperature of 37°C for lipase production by \textit{Bacillus} sp.\textsuperscript{32} and \textit{Bacillus} sp. MPTK 91\textsuperscript{33} isolated from oil mill effluent. Thermophilic lipases have a great potential in the detergent and food industries\textsuperscript{34} and, therefore, the studied organism may be exploited and scaling-up could be attempted for industrial production.

**Effect of carbon and nitrogen sources on lipase activity**

The carbon and nitrogen sources play a crucial role in the enzyme induction in bacteria\textsuperscript{35}. The major factor for the expression of lipase activity has always been carbon, since lipases are inducible enzymes and are thus generally produced in the presence of a lipid source or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, tweens, etc. Various carbon, nitrogen sources and metal ions were used for the production of lipase by \textit{B. stearothermophilus}.
isolated from olive oil mill soil. When glucose in basal medium was replaced by various sugars (lactose, galactose, sucrose, starch xylose, sorbitol or mannitol), xylose was the best source for lipase production (Fig. 3A). Xylose (875 ± 21.21 U/ml) increased the production of lipase by 24%, when compared to control (glucose, 705 ± 7.07 U/ml). The lipase production was affected by carbon sources in the following order: xylose > sorbitol > glucose> mannitol > galactose > lactose > sucrose > starch.

The effect of different nitrogen sources on lipase production by *B. stearothermophilus* strain revealed the highest lipase production (758.5 ± 30.4 U/mL) with nitrogen source peptone supplemented medium (Fig. 3B). Earlier studies have also shown the maximum lipase production by various *Bacillus* sp. in peptone supplemented medium.

**Effect of triglycerides and surfactants on lipase activity**

Besides carbon and nitrogen sources, among the different substrates tested, olive oil was found to be suitable for enhancing the lipase production by the isolated *B. stearothermophilus* strain and the maximum lipase activity (1460.5 ± 32.52 U/ml) was recorded (Fig. 4A). Our findings collaborated with previous findings.

The effect of detergents on lipase activity showed a low enzyme activity when SDS was added to the production medium, compared to other detergents (Fig. 4B). Tween-80 and to lesser extent triton-100 and tween-20 esters were shown to enhance lipase production after 48 h of incubation (1812.5 ± 41.01 U/ml). The lipase production significantly decreased when CHAPS and PEG-200 were used (Fig. 4B). The study was in line with the earlier reports which have found a total loss of activity in the presence of SDS, but in contrast, activity is enhanced in the presence of Triton X-100 and Tween esters. The higher level of lipase production has been observed, when the substrate forms an emulsion, thereby presenting an interfacial area to the enzyme.

![Fig. 3—Extracellular lipase production by *B. stearothermophilus* in various carbon (A) or nitrogen sources (B) after 48 h of growth.](image)

![Fig. 4—Influence of different lipids (A), surfactant compounds (B) and metal ions (C) on extracellular lipase production by *B. stearothermophilus* strain after 48 h of growth.](image)
The metal ions in media are an important factor that affects the enzyme production due to their acting as inducers. The effect of different metal ions on lipase production by *B. stearothermophilus* strain is shown in Fig. 5C. In the present study, maximum (2381.75 ± 73.5 U/mL) lipase production was observed in MgSO$_4$ supplemented medium, followed by MgCl$_2$. Earlier, maximum lipase production by *Bacillus coagulans* and *Bacillus cereus* strain MSU AS$^{27}$ has been reported with Ca$^{2+}$ and Mg$^{2+}$ ions. The Ca$^{2+}$ also increases lipase production by *Pseudomonas fluorescens* 2D$^{48}$ and *Antrodia cinnamomea*. Mg$^{2+}$ and Zn$^{2+}$ ions are also reported to significantly increase lipase production by *Enterobacter agglomerans*. $^{49}$

**Purification of Bacillus lipase**

Most of the commercial applications of the enzymes do not always require homogeneous preparation of the enzyme. However, a certain degree of purity is required, depending upon the final application in industries, such as fine chemicals, pharmaceuticals and cosmetics. Besides, purification of the enzyme is important for understanding the 3-D structure and the structure-function relationships of proteins. For industrial purposes, the purification strategies employed should be inexpensive, rapid, high-yielding and amenable to large-scale operations. They should have the potential for continuous product recovery with a relatively high capacity and selectivity for the desired product.

In the present study, BL1 was purified 50-fold with 61% yield by a single step of CM-Sephadex chromatography in (Fig. 5A, Table 2). The purity was confirmed by the presence of a single band on non-denaturing PAGE gels and molecular mass of BL1 was determined by SDS-PAGE to be 67 kDa (Fig. 5B). Specific activity of the purified enzyme reached 7850 U/mg, when olive emulsion was used as a substrate at pH 11, 55°C and in the presence of 1 mM NaDC and 1 mM CaCl$_2$ (Table 2). Interestingly, pure BL1 showed much higher specific activity than that displayed by *Bacillus* sp. MPTK 912 (3.7 U/mg) in olive oil emulsion$^{33}$.

Purified lipase was denatured, reduced and alkylated as described above and dialyzed against distilled water. The NH$_2$-terminal sequencing showed unambiguously the identification of 34 residues of the pure enzyme: ASIRA NDVPI VLLHG FTGWG KEEMF GFFYW GGLR. The N-terminal sequence of isolated lipase exhibited a high degree of similarity (>94%) and identity (>82%) with those of *Bacillus* sp. described so far$^{50,53}$.  

**Characterization of purified Bacillus lipase**

**Effect of pH and temperature on the activity and stability of lipase**

A great deal of research is currently going into developing lipases that can work under alkaline conditions as fat stain removers. The purified BL1 in the present study had better stability and activity at

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total activity (units$^{(a)}$)</th>
<th>Protein (mg$^{(b)}$)</th>
<th>Specific activity (U/mg)</th>
<th>Activity recovery (%)</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture supernatant</td>
<td>22075</td>
<td>140.6</td>
<td>157</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Acid and heat treatment</td>
<td>17854</td>
<td>17.3</td>
<td>1030</td>
<td>81</td>
<td>6.56</td>
</tr>
<tr>
<td>CM-Sephadex</td>
<td>13460</td>
<td>1.17</td>
<td>7850</td>
<td>61</td>
<td>50</td>
</tr>
</tbody>
</table>

$^{(a)}$1 Unit: umole of fatty acid released per min using olive oil emulsion as a substrate in the presence of 1 mM NaDC and 1 mM CaCl$_2$.

$^{(b)}$Proteins were estimated using the Bradford method$^{17}$. The experiments were conducted three times.
high temperature and alkaline pH. In fact, purified enzyme showed a very high alkaline pH optimum equal to 11 and was more stable over wider pH range than the *Bacillus* lipases studied before with more than 70% activity retention in the pH range 3-13. Furthermore, the activity increased significantly with increasing the temperature to reach its maximum value at 55°C. In temperature stability study, BL1 retained 90% or 60% of its activity after 60 min incubation at 55 or 75°C, respectively (Fig. 6). Thus, attractive characteristics, activity and stability at high temperature and alkaline conditions suggested its usefulness in detergent applications.

Kinetic study

BL1 hydrolyzed both short and long-chain triacylglycerols at comparable rates (Table 3). It showed specific activity of 7900, 7750 and 7430 U/mg using TC<sub>18</sub>, TC<sub>8</sub> and TC<sub>4</sub> as substrates, respectively at pH 11 and at 55°C in the presence of 1 mM CaCl<sub>2</sub> and 1 mM NaDC. Kinetic constants *Km*<sub>app</sub>, *kcat* and the deduced catalytic efficiency (*kcat*/*Km*<sub>app</sub>) of the purified lipase were determined and compared using Lineweaver-Burk plots using pure TC<sub>4</sub>, TC<sub>8</sub> or TC<sub>18</sub> as substrates under optimal conditions (Table 3). The *Km*<sub>app</sub> and *kcat* values were comparable even with the increase in aliphatic chain length of triacylglycerols. The deduced value of (*kcat*/*Km*<sub>app</sub>) of BL1 lipase was 1796.07, 1716.6, and 1586.06 using TC<sub>18</sub>, TC<sub>8</sub> or TC<sub>4</sub> as substrates, respectively. These results indicated that the lipase hydrolyzed both long and short-chain triacylglycerols at comparable rates and thus was a “true” lipase (Table 3). Earlier, lipases from *Bacillus thermocatenulatus* and *Staphylococcus* sp. have been reported to hydrolyse TC<sub>4</sub> preferentially<sup>55,56</sup>. It is also demonstrated that *Bacillus subtilis* Pa2 lipase is more active towards lipids containing C-18 unsaturated fatty acid and shows increase in the activity with increase in the percentage of unsaturated fatty acid in the oils<sup>57</sup>.

Effect of surfactants, oxidizing agents and detergents on lipase activity

For effective use under harsh detergent industry conditions, lipolytic enzyme must be compatible and stable with all commonly used detergent formulation ingredients, such as surfactants and oxidizing agent<sup>58</sup>. Results in Table 4 showed that BL1 was stable in several surfactants and retained 104, 110, 97, 85, 109 and 105% of its control activity in the presence of Tween-20, Tween-80, Triton X-100, SDS, sodium cholate and sodium taurocholate, respectively. It also retained 86, 90 and 82% of its activity after exposure to hydrogen peroxide, sodium hypochlorite and sodium perborate, respectively (Table 4), indicating that it was stable against oxidizing agents. Furthermore, the enzyme had very little loss in the activity after exposure to various commercial detergents (Table 4), indicating that it could be used as an additive to these indigenous detergents.

<table>
<thead>
<tr>
<th>Substrate</th>
<th><em>Km</em>&lt;sub&gt;app&lt;/sub&gt; (mM)</th>
<th><em>V&lt;sub&gt;max&lt;/sub&gt;</em> (mol/min/mg)</th>
<th><em>kcat</em> (s&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th><em>kcat</em>/<em>Km</em>&lt;sub&gt;app&lt;/sub&gt; (s&lt;sup&gt;-1&lt;/sup&gt; mM&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<td>4.92</td>
<td>7900</td>
<td>8836.69</td>
<td>1796.07</td>
</tr>
</tbody>
</table>

Table 3—Chain length selectivity and kinetic parameters of the purified BL1

![Fig 6](A) Effect of temperature on the activity (□) and stability (■) of BL1; (B) Effect of pH on the activity (□) and the stability (■) of BL1.
Effect of organic solvents on lipase stability

Organic solvents have been found to be advantageous in various industrial enzymatic processes and their use can increase the solubility of non-polar substrates, increase the thermal stability of enzymes, decrease water-dependent side reactions, or eliminate microbial contamination. As shown in Table 5, BL1 was also found to be fairly stable in the presence of water miscible organic solvents (Table 5). It retained almost 100% of its activity after incubation for 2 h at 25°C with 50% ethanol, 50% 2-propanol, 50% heptane and 50% cyclohexane (Table 5). Addition of 50% hexane to purified BL1 caused immediate increase of 15% in activity, as compared to the controls (Table 5). Thus, a high stability in the presence of organic solvents suggested that it could be considered a potential possible candidate for future applications in the detergent industry.

Conclusion

A new Bacillus stearothermophilus strain that produces alkaline lipase at high temperature was isolated from an olive oil contaminated soil. The purified extracellular lipase showed a high activity and stability in high alkaline pH and high temperatures. It showed stability not only towards the non-ionic surfactants like Triton X-100 and Tween-20, but also towards the strong anionic surfactant, SDS and oxidizing agents. Furthermore, the enzyme exhibited a high stability in the presence of organic solvents and various commercial laundry detergents. Thus, BL1 could be considered as a potential candidate to be used in biotechnology and essentially for application in the detergent industry.

Acknowledgments

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References


| Table 4—BL1 stability in presence of surfactants, detergents and oxidizing agents [Lipase preparation was incubated with different detergents components for 1 h at 40°C and the remaining activity was measured under standard assay conditions. Residual lipase activity was determined at pH 11 and 55°C. Results are the relative lipase activity expressed as the percentage of the maximum activity recorded without the addition of compound. Data are means of triplicate determinations ± standard deviation] |
|---|---|
| Detergent components (1%) | Residual activity (%) |
| None | 100±0.7 |
| **Surfactants** | |
| Tween-20 | 104±1.2 |
| Tween-80 | 110±1.5 |
| Triton–X100 | 97±0.5 |
| SDS | 85±1 |
| Sodium cholate | 109±2 |
| Sodium taurocholate | 105±1.5 |
| **Oxidizing agents** | |
| Hydrogen peroxide | 86±0.6 |
| Sodium perborate | 82±1.2 |
| Sodium hypochlorite | 90±0.8 |
| **Commercial detergents** | |
| Ariel | 92±1.2 |
| OMO | 90±1 |
| Tide | 87±2.3 |
| Daxon | 95±0.6 |

| Table 5—Stability of BL1 in organic solvents [Pure lipase samples (1 mg/ml) were incubated in each organic solvent (50%) at 25°C for 1 and 2 h. The remaining lipase activity relative to the non-solvent containing control was measured on tributyrin substrate. Values are average of assays carried out in triplicate] |
|---|---|---|
| Organic solvent | Relative activity% (1 h) | Relative activity% (2 h) |
| Control | 100 ± 0.5 | 100 ± 0.5 |
| Acetone | 95 ± 2.5 | 90 ± 2.9 |
| Acetonitrile | 92 ± 3.5 | 87 ± 2.4 |
| Methanol | 85 ± 3.6 | 82 ± 2.8 |
| Ethanol | 106 ± 3.7 | 100 ± 1.9 |
| Propanol | 94 ± 3.5 | 92 ± 4.2 |
| Hexane | 115 ± 1.5 | 110 ± 3.5 |
| Heptane | 109 ± 4.5 | 100 ± 2.5 |
| Cyclohexane | 110 ± 2.5 | 102 ± 1.5 |