Purification and characterisation of a thermostable alkaline lipase from a new thermophilic Bacillus sp. RSJ-1

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Abstract

An extracellular alkaline lipase from a new thermophilic Bacillus sp. RSJ-1 was purified to homogeneity by ultrafiltration, followed by ammonium sulfate precipitation, dialysis, Q-Sepharose ion exchange chromatography and Sephacryl S-200 SF gel filtration chromatography. This purification protocol resulted in a 201-fold purification of lipase with 19.7% final yield and the relative molecular weight of the enzyme was determined to be 37 kDa by SDS-PAGE. The kinetic characterisation of the purified enzyme exhibited maximum activity at 50 °C and pH 8.0–9.0. It was stable at 50 °C for 60 min and retained > 90% of its original activity for 120 min. The enzyme was also highly stable in a pH range of 8.0–9.0 for 120 min. The enzyme activity was promoted in the presence of Ca2+, Na+, Mg2+ and Ba2+ and was strongly inhibited by Cs+, K+, Co2+ and Zn2+. EDTA did not affect the enzyme activity, whereas the presence of various oxidizing agents, reducing agents and some surfactants, reduced the enzyme activity. The enzyme was highly stable in the presence of some commercial detergent formulations. The values of $K_m$ and $V_{max}$, as calculated from the Lineweaver–Burk plot, were 2.2 mg/ml and 1429 U/ml, respectively. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Alkaline; Lipase; Thermophilic; Bacillus sp; Purification; Thermostable

1. Introduction

Lipases (triacylglycerol acyl hydrolases (E.C. 3.1.1.3)) are one of the most important classes of hydrolytic enzymes that catalyze both the hydrolysis and the synthesis of esters. Lipases have a number of unique characteristics, including substrate specificity, stereospecificity, regioselectivity and the ability to catalyze a heterogenous reaction at the interface of water soluble and water insoluble systems [1]. Lipases are ubiquitous enzymes produced by all biological systems, viz. animals, plants and microorganisms. In contrast to animal and plant lipases, extracellular microbial lipases can be produced relatively inexpensively by fermentation and in large quantities [2].

With the rapid development of enzyme technology, many new potential biotechnological applications for lipases have been identified in the areas of detergent industry, oleochemical industry, food industry, organic synthetic industry, paper manufacturing industry, biosurfactant synthesis, cosmetics and pharmaceuticals [3–5]. As the applications increase, the availability of lipase possessing satisfactory operating characteristics is a limiting factor. Lipase used in detergents needs to be stable under alkaline pH and should be active in the presence of surfactants. As most of the industrial processes operate at a temperature exceeding 45 °C, lipase should be active and stable at a temperature around 50 °C. Hence, alkalophilic and thermophilic microorganisms have been the focus of a number of investigations into the sources of lipases that are stable and function optimally at extreme alkaline pH values and high temperature. Isolates of Bacillus sp. have been found to produce lipolytic enzymes under alkaline conditions [6]. Lipases from Bacillus subtilis and B. pumilis [7] have been of particular interest, as they exhibit optimal activity and stability at extreme alkaline pH values > 9.5. These enzymes are, however, thermolabile. An-
other lipase produced by a recombinant *B. licheniformis* showed maximum activity at pH 10–11.5 and was remarkably stable at alkaline pH values up to 12.0 [8], but was mildly thermotolerant, retaining only 25% activity after 30 min incubation at 50 °C. These results are in contrast to lipases from *B. thermoacetogenes* [9] and *B. thermoacetenulatus* [10] which are thermostable, but display maximum activity and stability at moderate alkaline pH values (7.0–8.5).

In view of the above, we conducted an extensive screening and isolated a new thermophilic alkaline lipase producing organism, identified as *Bacillus* sp. RSJ-1. Here, we report the purification and characterisation of its thermostable alkaline lipase.

### 2. Materials and methods

#### 2.1. Bacterial strain and cultural conditions

The thermophilic *Bacillus* sp. RSJ-1, used in the present study, was isolated from the hot springs of Manikaran, India at 50 °C and pH 9.0 and identified according to Bergey’s Manual of Systematic Bacteriology [11]. The organism was Gram positive, rod-shaped, encapsulated, spore former and exhibited the typical morphological, physiological and biochemical characteristics of genus *Bacillus*. It was a true thermophile and could grow up to 65 °C with a pH optimum of 9.0 for growth. It could not match fully with a known species and thus was tentatively named as a new *Bacillus* sp. RSJ-1.

Cells were grown on tributyrin agar plates (0.3% meat extract, 0.5% peptone, 0.9% NaCl, 0.25% tributyrin, 0.001% CaCl₂·2H₂O) and then incubated in 250 ml Erlenmeyer flasks, each containing 50 ml of nutrient broth and then incubated for 10 h in a New Brunswick waterbath shaker (200 rpm) at 50 °C to raise the inoculum for the enzyme production. Seed culture (150 ml), having a cell count of 10⁸/ml, was passed through a 10 kDa membrane (Sartorius GmbH 3400 Gottingen, Germany) and one-fifth of the retentate was collected. To the ultrafiltration retentate, ammonium sulfate was slowly added to attain 70% saturation at 4 °C. The precipitates formed were separated by centrifugation (14,000 × g for 10 min at 4 °C). These precipitates were then dissolved in 100 mM Tris–HCl, pH 8.0 and dialyzed against 50 mM Tris–HCl buffer, pH 8.0 and kept at 4 °C for 18 h. The dialyzed material was centrifuged (12,000 × g for 20 min at 4 °C) and the supernatant was retained for further purification by Q-Sepharose followed by Sephacryl S-200 SF column chromatography.

#### 2.4.1. Q-Sepharose column chromatography

The supernatant after dialysis containing enzyme protein was applied to a Q-Sepharose column (1.4 × 10 cm, 5 ml of gel) which had been equilibrated with 50 mM Tris–HCl buffer, pH 8.0 (Buffer A). The lipase was eluted at a flow rate of 60 ml/h by 1.0 vol of 1 M NaCl, (Buffer B) forming a gradient in the buffer (Buffer A). The fractions (1.0 ml) were collected and analyzed for lipase activity. The active fractions were pooled and concentrated to 1.0 ml by ultrafiltration in 25 kDa centricones (Amicon, USA).

#### 2.4.2. Sephacryl S-200 SF column chromatography

The concentrated sample obtained after Q-Sepharose column chromatography (0.5 ml) was then applied on a Sephacryl S-200 SF column (1.4 × 50 cm, 70 ml of gel) which was pre-equilibrated with 50 mM Tris–HCl buffer containing 1 mM calcium chloride and 150 mM sodium chloride, pH 8.0, at a flow rate of 30 ml/h. The enzyme was eluted with the same buffer. The fractions (1.0 ml) were collected and assayed for lipase activity. The active fractions were pooled, concentrated and analyzed for purity by SDS-PAGE.

#### 2.4.3. Electrophoresis

SDS-PAGE was performed on 15% polyacrylamide...
slab gels with 0.1% sodium dodecyl sulfate (SDS) to establish the purity of the enzyme protein, as described by Laemmli [15]. The native gels were run without SDS. For estimation of relative molecular weight 15% SDS-PAGE was run along with the following standards—phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soyabean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

2.5. Kinetic characterisation

The kinetic studies on lipase obtained after Q-Sepharose column chromatography was carried out in terms of optimum temperature, thermostability, optimum pH and pH stability, metal ions, oxidizing, reducing and chelating agents. The enzyme was also studied for Michaelis–Menten constants, $K_m$ and $V_{max}$. The enzyme assays for all the experiments except the effect of pH were carried out at pH 8.0 by a spectrophotometric method using pNPB as the substrate. However, in the case of pH studies, enzyme assays were performed by a cupric acetate method.

2.5.1. Effect of temperature

The effect of temperature on lipase activity was studied by carrying out the enzyme reaction at different temperatures in the range of 40–90 °C at pH 8.0 using Tris–HCl buffer (50 mM). The thermostability of lipase was tested by preincubating at varying temperatures ranging from 50 to 85 °C for different time intervals from 0 to 240 min.

2.5.2. Effect of pH

pH activity and stability profiles were studied by a cupric acetate assay procedure in a pH range of 5.0–12.0 using different buffers at 50 mM concentration: citrate–phosphate buffer (pH 4.0–6.0), Tris–HCl buffer (pH 7.0–8.0), carbonate–bicarbonate buffer (pH 9.0–10.0) and glycine–NaOH buffer (pH 11.0–12.0). For stability studies, 1 vol of enzyme was mixed with 4 vol of respective buffer and incubated up to 120 min.

2.5.3. Effect of effector molecules on lipase activity

The effect of various metal ions, oxidizing, reducing and chelating agents was studied at a concentration of 1 mM at 50 °C and pH 8.0.

2.5.4. Determination of Michaelis–Menten constants

Enzyme assays with 100 µl of purified lipase were performed in Tris–HCl buffer, pH 8.0 at 50 °C with increasing concentration of p-nitrophenyl butrate (pNPB) from 0.3 to 4.0 mg/ml. Lineweaver–Burk plot was plotted to determine $K_m$ and $V_{max}$.

2.6. Evaluation of enzyme for use in detergent formulations

In order to determine the suitability of enzyme for use in detergents, its compatibility was studied in various detergent ingredients including surfactants, hydrogen peroxide and commercial detergents, by directly incorporating these into the assay mixture.

2.6.1. Stability of the enzyme preparation in the presence of commercial detergent

Since the lipase retained maximum activity in the presence of Advanced Ariel Compact detergent powder under normal assay conditions when added directly to the assay mixture, the stability of lipase in this detergent powder was studied at 50 and 60 °C in the presence of 1 mM calcium chloride as stabilizer.

The detergent was added to the enzyme in the concentration of 10 mg/3 ml and incubated at 50 and 60 °C. Samples (100 µl) of lipase were withdrawn at regular intervals of 10 min up to 1 h. The residual lipase activity in each sample was determined under standard assay conditions.

3. Results and discussion

3.1. Purification of lipase

In our attempt to purify lipase, the cell free supernatant, concentrated by ultrafiltration, was subjected to ammonium sulfate precipitation, ion exchange chromatography on Q-Sepharose followed by gel filtration on Sephacryl S-200 column. The lipase was finally purified 201-fold (Table 1; Figs. 1 and 2). The purified lipase was homogenous when tested by Coomassie blue staining of SDS-PAGE and had a molecular weight of 37 kDa (Plate 1). Silver staining also showed the same results. The lower molecular weight of the lipase is advantageous, as smaller enzymes are more stable due to smaller changes (unfolding) in tertiary structure [16]. Wang et al. [17] purified an alkaline thermostable lipase from Bacillus sp. [18] was purified to homogeneity by extraction, Bio-gel P-10 chromatography and Superose 12 B chromatography and a 37-fold purification was attained. The purified enzyme showed a single band with a molecular weight of 30 kDa when it was sub-
jected to SDS-PAGE. Chahinian et al. [19] also purified a lipase from *Penicillium cyclopium* by ammonium sulfate precipitation and chromatographies on Sephadex G-75 and DEAE Sephadex. The enzyme isolated in several glycosylated forms of 40–43 kDa, which could be converted to a single protein of 37 kDa by enzymic deglycosylation.

From the above discussion it can be seen that a three- to four-step purification protocol was followed by most of the workers, but compared to others, the lipase obtained from *Bacillus* sp. RSJ-1 was purified 201-fold with a high specific activity (428 U/mg) using a simple purification protocol.

### 3.2. Characterisation of purified lipase

Thermal energy, unlike extremes of pH and low water activity, penetrates across the cell envelope. Therefore, cellular components of thermophiles have adapted to function at high temperatures [20]. It is the activity and stability of cellular components, such as proteins, ribosomes, nucleic acids, membranes at high temperatures, forms the basis of thermostability of proteins. Recent advances in the study of extremozymes have led to the acceleration of use of these in diverse industrial applications. In particular, enzymes from thermophilic

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Step</th>
<th>Volume (ml)</th>
<th>Total activity (U)</th>
<th>Total protein (mg)</th>
<th>Specific activity (U/mg)</th>
<th>Yield (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crude (cell free supernatant)</td>
<td>500</td>
<td>5100</td>
<td>2400</td>
<td>2.13</td>
<td>100.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>Ultrafiltration (10 kDa)</td>
<td>100</td>
<td>4050</td>
<td>450</td>
<td>9.00</td>
<td>79.40</td>
<td>4.20</td>
</tr>
<tr>
<td>3</td>
<td>Precipitation (NH₄)₂SO₄ (30–70%)</td>
<td>40</td>
<td>2425</td>
<td>205</td>
<td>11.82</td>
<td>47.50</td>
<td>5.56</td>
</tr>
<tr>
<td>4</td>
<td>Dialysis</td>
<td>30</td>
<td>1824</td>
<td>68.2</td>
<td>26.74</td>
<td>35.76</td>
<td>12.50</td>
</tr>
<tr>
<td>5</td>
<td>Ion exchange</td>
<td>10</td>
<td>1432</td>
<td>14.0</td>
<td>102.28</td>
<td>28.07</td>
<td>48.13</td>
</tr>
<tr>
<td>6</td>
<td>Gel filtration</td>
<td>10</td>
<td>1006</td>
<td>2.35</td>
<td>428.08</td>
<td>19.70</td>
<td>201.45</td>
</tr>
</tbody>
</table>

**Fig. 1.** Purification profile of lipase from *Bacillus* sp. RSJ-1 on Q-Sepharose ion exchange chromatography. Column (1.4 × 10 cm, 5 ml) was equilibrated with 50 mM Tris–HCl buffer, pH 8.0. The lipase was eluted with a gradient of sodium chloride in 50 mM Tris–HCl buffer, pH 8.0.
organisms have found the most practical commercial use to date because of their overall inherent stability [21]. Also, since most of the industrial processes involving enzymes operate at temperatures above 50 °C, the importance of thermostable enzymes is of great significance. The lipase from *Bacillus* sp. RSJ-1 was thermostable as indicated by the optimum temperature of the activity of pure lipase, which was 50 °C (Fig. 3). The enzyme retained 96, 92, 78 and 34%, of its maximum activity at 55, 60, 65 and 70 °C, respectively. The purified lipase retained more than 90 and 70% of its original activity for 120 and 240 min, respectively at 50 °C, but the half life of the enzyme was 150, 90, 55 and 45 min at 60, 65, 70 and 75 °C, respectively (Fig. 4).

The stability of thermophilic proteins is intrinsic and resides in their primary structure. Thermostabilization of proteins is achieved through optimization of intramolecular interactions, packing densities, internalization of hydrophobic residues and surface exposure of hydrophilic residues [20]. Any approach for stabilizing an enzyme, that is for preventing or at least slowing down, its inactivation may be directed either towards displacing the N ↔ U equilibrium (i.e. by stabilizing the native state relative to the unfolded) or towards preventing the essentially irreversible process which occurs after U is produced [16]. A number of soluble additives to stabilize the enzyme have also been used which may be substrates, low molecular weight organic solutes, salts, polymeric solutes and synthetic polymers [22]. Other workers have found optimum temperature for

![Fig. 2. Purification profile of lipase from *Bacillus* sp. RSJ-1 on Sephacryl S-200 SF gel filtration chromatography. Column (1.4 × 50 cm, 70 ml) was equilibrated with 50 mM Tris–HCl buffer, pH 8.0 containing 150 mM sodium chloride and 1 mM calcium chloride at a flow rate of 30 ml/h. The sample 0.5 ml was loaded and eluted with the same buffer.](image1)

![Plate 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis pattern of purified lipase from *Bacillus* sp. RSJ-1. SDS-PAGE was conducted in 15% gel and the protein was stained with Coomassie blue R 250. Lane A: purified lipase from *Bacillus* sp. RSJ-1. Lane B: standard proteins (with molecular weights in brackets) are phosphorlyase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soyabean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa). Side panel in the plate indicates the molecular weight of respective proteins in kDa.](image2)
Fig. 3. Effect of temperature and pH on lipase activity from *Bacillus* sp. RSJ-1.

Fig. 4. Thermostability profile of purified lipase from *Bacillus* sp. RSJ-1.

thermostable *Bacillus* sp. lipase to be 50–55 and 60 °C [17,23]. The thermostability of the present lipase preparation was comparable to that of recently reported *B. stearothermophilus* [24] which was stable up to 55 °C for 30 min and the one reported by Sugihara et al. [25] from *Bacillus* sp. where lipase was stable up to 65 °C for 30 min. However, highly thermostable lipase was reported by Wang et al. [17] from *Bacillus* strain A30-1 (ATCC 53841) that retained 100% of the original activity after being heated at 75 °C for 30 min, while the half life at 75 °C was 8 h. In purified *Bacillus* sp. RSJ-1, as the half life of the enzyme has been observed to be 150 min at 60 °C, the effect of different additives on the thermostability of the purified enzyme was also checked. In the case of CaCl$_2$·2H$_2$O (1 mM) the half life of the lipase increased to 210 min. As the concentration of CaCl$_2$·2H$_2$O increased from 1–10 mM, the stabilizing effect decreased (data not shown). Whereas 100% activity was observed at 1 mM, it decreased to 4% of the maximum activity at 10 mM concentration.

The protein nature of enzymes means that pH will affect the ionization state of the amino acids which dictate the primary and secondary structure of the enzyme and hence, controls its overall activity. A change in pH will have a progressive effect on the structure of the protein and the enzyme activity [26]. Several workers found optimal lipase activity in the highly alkaline pH range (8.5–10.0) from *Bacillus* strain A30-1 (ATCC 53841) [17] and *B. stearothermophilus* L1 [27]. A purified lipase from *P. pseudoalcaligenes* F-111 [13] was found to be stable in a pH range between 6.0 and 10.0 and Sharon et al. [28] also reported a lipase produced by *P. aeruginosa* KKA-5, which was stable in a pH range from 7 to 10 for 24 h at 30 °C.

The lipase from *Bacillus* sp. RSJ-1 showed activity in a very wide pH range (5.0–12.0). Maximal activity (100%) was observed at pH 8.0 (Fig. 3), while this was closely followed by pH 9.0 (99% of the maximum). The activity reduced drastically at pH 5.0 (12%) and was 18% of the maximum at pH 12.0. The enzyme showed good stability after 2 h in an alkaline pH range, where the enzyme retained 84 and 82% of the maximum activity at pH 8.0 and 9.0, respectively but was totally inactivated at acidic pH (5.0) and lost 96% of its original activity at pH 12.0 (Fig. 5).

Metal ions and salts are of importance in thermostable enzymes from thermophilic organisms. Stabilization of enzymes by metal ions at high temperatures is by metal ion complexation, which is a process with a favorable entropy factor [16]. This is because water previously bound to the hydrated metal ion in solution is liberated when the metal ion becomes bound to the protein. Thus, the process is favored at high temperatures. A number of enzymes require the presence of metal ions, such as calcium ions, for the maintenance of their stable and active structures. These ions are bound strongly to specific binding sites on the surface of the molecules. The binding sites are usually constructed from negatively charged carboxylate side-chain groups of aspartyl and glutamyl residues, brought together by folding of the polypeptide chain [16]. Dissociation constants for the binding are low (of the order of $10^{-3}$ to $10^{-6}$ M) in the case of E + Ca$^2+$ ⇋ E$^{-}$ Ca$^{2+}$, represent-
ing very strong binding and also emphasizing that the effects took place at low calcium ion concentrations. This phenomenon supports our results.

As can be seen from the above equation, the situation is similar to that of enzyme-substrate complexes. The polypeptide chain is 'cross linked' by the metal ions bridge and the enzyme–calcium ion complex should, therefore, be more rigid and hence more stable [16]. The bridging by metal ions in this way is compared to that brought about by disulfide formation. In the absence of calcium ions, the binding site would represent a high local concentration of negative charges. The tendency of these groups to move apart to reduce the repulsive electrostatic interactions would contribute to the relative instability of the folded protein. A total of 1 mM CaCl$_2$·2H$_2$O also enhanced activity by 16% when added as a metal ion. The lipase activity in the enzyme preparation from *Bacillus* sp. RSJ-1 was also promoted in the presence of Na$^+$, Mg$^{2+}$ and Ba$^{2+}$ (at 1 mM concentration) by 12, 2 and 2% respectively. It was strongly inhibited to 49% by Cs$^+$ and 47% each with K$^+$, Co$^{2+}$ and Zn$^{2+}$ (Table 2).

The activity of the lipase from *Bacillus* sp. RSJ-1 decreased with both oxidizing and reducing agents (Table 2). Among the oxidizing agents, the enzyme retained 72% activity with ammonium persulfate, while with potassium iodide, it retained 47% of the maximum activity. When reducing agents ascorbic acid and 2-mercaptoethanol were added at 1 mM concentration, enzyme activity fell by 26 and 36%, respectively. Some 1 mM EDTA did not affect the enzyme activity indicating that the lipase from *Bacillus* sp. RSJ-1 is not a metalloenzyme. Loss of activity (4%) was observed when another chelating agent, sodium citrate (1 mM) was added to the reaction mixture (Table 2). Lin et al. [13] also showed that activity of lipase produced from *P. pseudocallicligenes* F-111 was not affected by EDTA (1 mM).

The values of $K_m$ and $V_{max}$ of the purified lipase from *Bacillus* sp. RSJ-1, using pNPB as calculated from the Lineweaver–Burk plot, were 2.2 mg/ml and 1428.6 U/ml, respectively (Fig. 6). The $K_m$ values of the enzyme range widely, but for most industrially used enzymes, they lie in the range of $10^{-1}$ to $10^{-5}$ M when acting on biotechnologically important substrates [26].

### 3.3. Compatibility of lipase with detergents

In general, all detergent compatible enzymes are alkaline and thermostable in nature with a high pH optimum because the pH of laundry detergents is generally in the range of 9–12 and have varying thermostability at laundry temperatures of 50/60 °C [29].

#### Table 2

<table>
<thead>
<tr>
<th>Effector molecule</th>
<th>Relative activity (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metal salts</strong></td>
<td></td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>116</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>102</td>
</tr>
<tr>
<td>Cupric chloride</td>
<td>56</td>
</tr>
<tr>
<td>Sodium dihydrogen</td>
<td>91</td>
</tr>
<tr>
<td>orthophosphate</td>
<td></td>
</tr>
<tr>
<td>Cesium chloride</td>
<td>49</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>47</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>100</td>
</tr>
<tr>
<td>Cobaltous chloride</td>
<td>47</td>
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<td>Zinc chloride</td>
<td>47</td>
</tr>
<tr>
<td>Barium chloride</td>
<td>103</td>
</tr>
<tr>
<td>Stannous chloride</td>
<td>46</td>
</tr>
<tr>
<td>Manganese chloride</td>
<td>63</td>
</tr>
<tr>
<td>Nickel chloride</td>
<td>88</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>112</td>
</tr>
<tr>
<td><strong>Oxidizing agents</strong></td>
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</tr>
<tr>
<td>Ammonium persulfate</td>
<td>72</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>47</td>
</tr>
<tr>
<td><strong>Reducing agents</strong></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>74</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>64</td>
</tr>
<tr>
<td><strong>Chelating agents</strong></td>
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<tr>
<td>Sodium citrate</td>
<td>96</td>
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<tr>
<td>EDTA</td>
<td>100</td>
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</tbody>
</table>

Fig. 5. pH stability profile of purified lipase from *Bacillus* sp. RSJ-1.
The present enzyme has good thermostability as it retained > 90% activity at 60 °C for 1 h under alkaline conditions and also exhibited a half life of > 150 min at 60 °C and 45 min at 70 °C, respectively. The enzyme showed good stability in an alkaline pH range, as it retained > 95% activity at pH 9.0, which reduced to 78% at pH 10.0 after 1 h of incubation. However, few data are available on thermal and pH stability of lipase Lipomax (Genencor International), a well-established commercial detergent lipase, but the thermal-pH stability of our lipase preparation was higher than Subtilisin Carlsberg, a commercial detergent protease. Thus, the present lipase is very suitable, with regard to pH and temperature characteristics relevant to detergent formulation.

Besides pH and temperature stability, a good detergent lipase should also be stable in the presence of surfactants, bleaching agents and detergents. The lipase was quite stable in some non-ionic surfactants and retained 100, 92 and 82% of its maximum activity in the presence of Triton X-100, Tween-80 and Tween-20, respectively, but was less stable in anionic surfactants, deoxycholate, as it retained only 54% activity (Fig. 7a).

Bleach stability of the enzyme was checked in the presence of hydrogen peroxide. The data on peroxide inactivation showed that the enzyme was stable at lower concentration of H₂O₂, i.e. it retained 93 and 74% activity at 0.1 and 0.2% concentration of H₂O₂, respectively and only 47% activity at 1% (Fig. 7b). Information on the bleach stable behaviour of enzymes, which is an important property for the addition of these to detergents, is not generally available except for a very few reports [17].

The compatibility of Bacillus sp. RSJ-1 lipase in the presence of certain commercial detergents was shown to be good as the enzyme retained > 55% of its maximum activity in most of these. The lipase was found to be highly stable (99%) in the presence of Advanced Ariel Compact and 95% stable in Henko (Stain Champion) followed by Gain Super Soaker, Rin Super and Surf Excel giving a residual activity of 93, 68 and 63%, respectively (Fig. 7c). The stability profile of lipase at 50 and 60 °C in the presence of 1 mM CaCl₂·2H₂O and Advanced Ariel Compact showed that the lipase was stable for 30 min giving 79 and 64% residual activity at 50 and 60 °C, respectively (data not shown).

Considering the overall properties of different alkaline lipases of microbial origin and the alkaline lipase studied from our isolate, Bacillus sp. RSJ-1 is better as regards to pH and temperature stability, stability in the presence of surfactants, detergent compatibility and above all bleach stability, for a potential application in the detergent industry.
Fig. 7. Compatibility of lipase from Bacillus sp. RSJ-1 with different (a) surfactants (b) concentrations of H₂O₂ and (c) commercial detergents.

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