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Production and Characterization of a Halo-, Solvent-, Thermo-tolerant Alkaline Lipase by *Staphylococcus arlettae* JPBW-1, Isolated from Rock Salt Mine

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Abstract Studies on lipase production and characterization were carried out with a bacterial strain *Staphylococcus arlettae* JPBW-1 isolated from rock salt mine, Darang, HP, India. Higher lipase activity has been obtained using 10 % inoculum with 5 % of soybean oil as 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 a broad range of temperature (30–90 °C), pH (7.0–12.0) and NaCl concentration (0–20 %). It has 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^+, Co^{2+} and Fe^{2+} and stimulated by Mn^{2+}, Ca^{2+} and Hg^{2+}. 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 7.05 mM and 2.67 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.

Keywords Production · Characterization · *Staphylococcus arlettae* lipase · Submerged fermentation

Introduction

Lipases (triacylglycerol acylhydrolases (E.C.3.1.1.3) are a class of hydrolases which 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 [1]. 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 [2]. Among different microbial sources, bacterial lipases received much attention for their wider industrial usage due to their ability to function in extreme environments. Bacterial lipases are

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mostly inducible enzymes with requirement of triglycerides as inducers [3]. These are mostly extracellular in nature and are produced mainly through submerged fermentation (SmF).

SmF holds tremendous potential for the production of lipases due to usage of this crude one directly as a lipase source for industrial application [4, 5]. Lipase production through SmF is dependent upon a number of factors including carbon and nitrogen sources, pH, temperature, aeration and inoculum size. Selection of fermentation conditions for microbial lipase production is of great interest, since culture conditions influence the properties of the enzyme produced as well as ratio of extra cellular 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 [6, 7]. 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 also prerequisite since low-water systems based on organic solvents are necessary in order to provide conditions that favor the synthetic reaction over the normal hydrolytic reaction [8]. This has drawn 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 stability 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 various unexploited regions of the earth, as small numbers of bacterial strains producing thermophilic lipases have been reported in the last decade [9]. Recently, a screening of lipase activity was carried out on halophilic bacteria from the salt lake of Yuncheng, China, in this work reported a moderately halophilic strain LY7-8 [10]. Recent studies also reported lipolytic enzyme-producing thermophilic microorganism named *Bacillus thermoamylovorans*, isolated from a hot spring in Galicia (North Western Spain) [11].

In the present study, lipase production from *S. arlettae* through SmF has been carried out, and partial purification has been done to characterize its stability in presence of pH, temperature, salt conditions, organic solvents, surfactants, inhibitors and metal ions.

Material and Methods

Chemicals

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

Microorganism and Culture Maintenance

The bacterial strain, *Staphylococcus arlettae* JPBW-1 used in this study was isolated from a 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 2 weeks to maintain its viability.

Lipase Production Through SmF

SmF was carried out by seeding the inoculum size (2–12 %) in Erlenmeyer flasks (250 ml) containing 100 ml of the LB medium (10 g of 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 selection of 100 ml media are tabulated in Table 1. The effects of various carbon sources (coconut oil, olive oil, and soybean oil) were estimated in relation to enzyme activity. The contents of the flask were autoclaved at 121 °C for 20 min. The flasks were incubated at different temperature (30–60 °C) and agitation speed (50–200 rpm) for different incubation time intervals (1–24 h). After incubation, the fermentation medium was harvested by centrifugation at 6,314×*g* for 10 min at 4 °C. The supernatant was collected and subjected to estimate the lipase activity.

Lipase Assay and Protein Estimation

Lipase activity was assayed quantitatively using p-Nitrophenyl palmitate as the substrate according to the method described by Garlapati and Banerjee [12]. One unit of lipase activity was defined as the amount of enzyme that liberated 1 μmol of *P*-nitrophenol per minute under the assay conditions. Protein content of cell-free supernatant was determined according to modified Lowry method [13] using bovine serum albumin as standard.

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 6,987×*g* for10 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 h. Then the lipase assay and protein estimation was done.

Characterization of Lipase

The effect of pH on lipase activity was analyzed by carrying out assays at different pH ranging from 4 to 12 using different buffers (acetate, phosphate, Tris–HCl and glycine and NaOH)) of 0.5 M concentration at 37 °C using *p*-nitophenyl palmitate as substrate by incubating for 1 h in buffers in 1:1 ratio and then relative activity was calculated with respective to control. 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. The effect of NaCl concentration was determined by incubating the enzyme solution with different concentrations of NaCl (0–25 %) for 60 min at 35 °C and measured activity 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 h. The effect of

Table 1 Selection of amount of media in 250-ml conical flask for SmF

Vol. of media (ml)	Lipase activity (U/ml)
25	0.026±0.60
50	0.05±0.77
75	0.1±0.58
100	0.19±0.46
125	0.08±0.84
150	0.035±0.62

Experimental conditions: temperature, 30 °C; inoculum size, 10 %; incubation period, 3 h; agitation speed, 100 rpm

various surfactants on the lipase activity was investigated by pre-incubating the enzyme for 60 min at 35 °C in Tris buffer (50 mM, pH 8.0) containing 1 mM Tween 40, Tween 20 and Triton-X 100. The effect of various chloride salts of the metal ions (Co^{2+}, Ca^{2+}, K^{+}, Mg^{2+}, Hg^{2+}, Fe^{2+}, Mn^{2+} and Na^{+} and chemical reagents EDTA, *o*-phenanthroline, PMSF, guanidine thiocynate, β-6-mercaptoethanol on the lipase activity were examined with them after incubation of the enzyme with 1 mM concentration at 1:1 ratio for 1 h at 35 ° C and then, 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 effect of substrate concentration *p*-nitrophenyl palmitate (1–5 mM) on the reaction rate was assayed at pH 8 by using spectrophotometric method. The Michaelis–Menten constant (K_m) and the maximum velocity for the reaction (Vmax) were determined by Lineweaver–Burk plot.

Results and Discussions

Selection of Parameters for Lipase Production Through SmF

Selection of Incubation Temperature

For selection of suitable temperature for lipase production the temperature was varied from 25 °C to 50 °C, keeping the other process conditions as constant. It has been observed that the maximal lipase activity (0.216 U/ml) occurred at 35 °C (Fig. 1). Mohan et al. [14] found that the optimum temperature for lipase production by *Bacillus* sp. was 37 °C. Similarly, Walavalkar and Bapal [15] have reported that the lipase activity of *Staphylococcus* sp. was high at 37 °C. Thermophilic lipases have a great potential in the detergent and food industries, and therefore the organism may be exploited and scaling up could be attempted for industrial production.

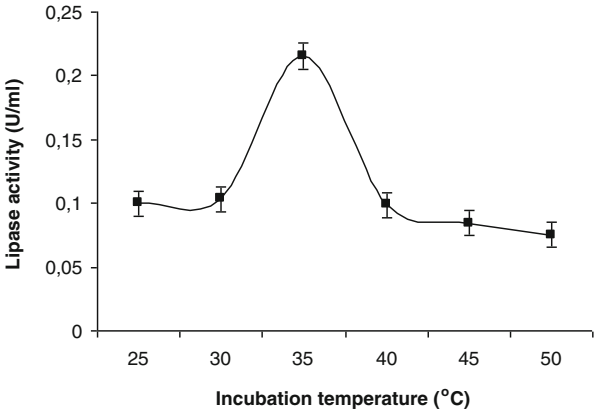

Fig. 1 Selection of incubation temperature for lipase production. All values are represented as ±SD of three replications. (Experimental conditions: inoculum size, 10 %; incubation period, 3 h; agitation speed, 100 rpm)

Selection of Inoculum Size

To evaluate the impact of inoculum size on lipase production by *S. arlettae*, experiments has been carried out 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 in 48 h. After a certain concentration, the lipase activity increased and then decreased (Fig. 2). This may be due to overpopulated culture and fixed amount of nutrient with which the organism starts liberating proteolytic enzyme, enhancing self consumption [16].

Selection of Media pH

The pH of the production medium plays a critical role in optimal physiological performances of bacterial cell and in nutrient transport across the cell membrane which plays an important role in acheiving higher 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. 3). A comprehensive review of all bacterial lipase done by Gupta et al. [17] states that maximum activity of lipases at pH values higher than 7 has been observed in many cases. Generally, bacterial lipases have neutral [18, 19] or alkaline pH optima [20–22], with the exception of *Pseudomonas fluorescens* SIK W1 lipase, which has an acidic optimum at pH 4.8 [23]

Selection of Incubation Time

The effect of incubation time on lipase activity was determined for 1–15 h, and it was noted that high lipase activity (0.628 U/ml) has been found with incubation time of 3 h (Fig. 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. An incubation period of 12 h was optimum for lipase production by *Bacillus* sp. RSJ1 [24] and 16 h for *B. thermocatenulatus* [25].

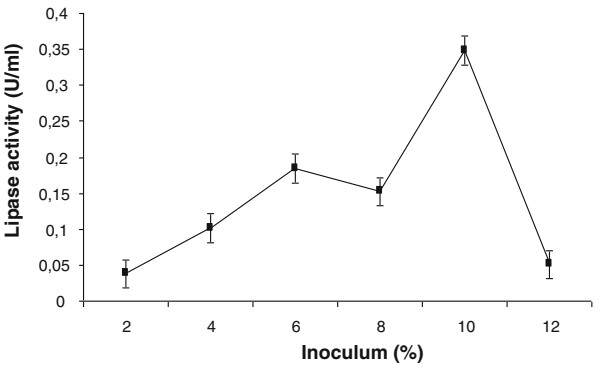

Fig. 2 Selection of inoculum size for lipase production. All values are represented as ±SD of three replications. (Experimental conditions: incubation period, 3 h; temperature, 35 °C; agitation speed, 100 rpm)

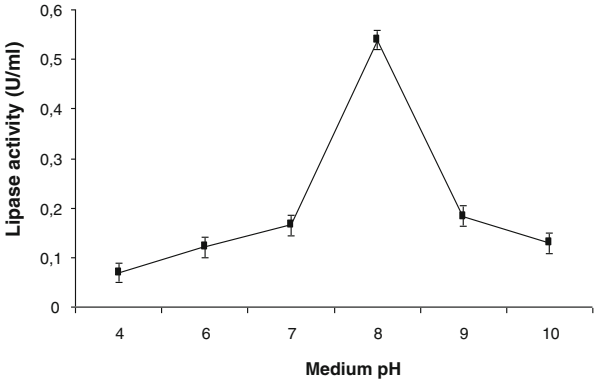

Fig. 3 Selection of pH of media for lipase production. All values are represented as ±SD of three replications. (Experimental conditions: inoculum size, 10 %; incubation period, 3 h; temperature, 35 °C)

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. 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 such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts and glycerol. 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. 5). This may be attributed to the substrate inhibition [26, 27].

Selection of Agitation Speed

To determine the optimal agitation speed for peak enzyme activity, the culture has been cultured in an orbital shaking incubator at 35 °C at varying agitation speeds from 50 to 200 rpm. 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

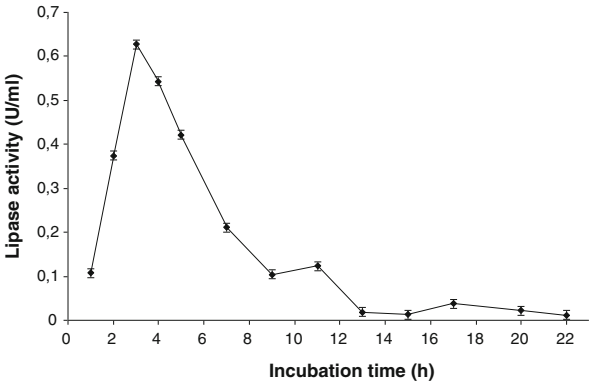

Fig. 4 Selection of Incubation time for lipase production. All values are represented as ±SD of three replications. (Experimental conditions: inoculum size, 10 %; temperature, 35 °C; agitation speed, 100 rpm; pH 8.0)

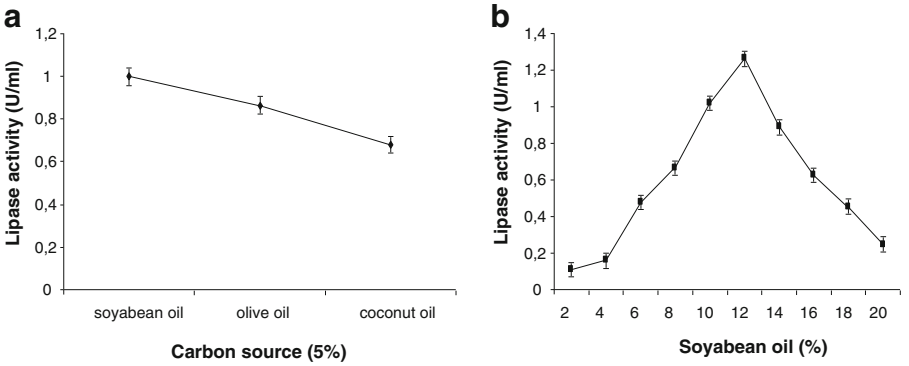

Fig. 5 Selection of carbon source (**a**) and its % (**b**) for lipase production. All values are represented as ±SD of three replications. (Experimental conditions: inoculum size, 10 %; incubation period, −3 h; temperature, 35 °C, pH 8.0)

same. Maximum lipase activity has been observed (2.162 U/ml) utilizing an agitation speed of 100 rpm (Fig. 6). From the results, the authors can interpret that the microorganism responsible for lipase production had a 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 on the negative effect of the higher mixing rates on the lipase production by *Geotrichum candidum.* Alford and Smith [28] reported that the lipase yields reduced for 60 % as a result of the mixing at low rates and even more at the higher rates. Wouters [29], similar 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.

Partial Purification of Lipase by Ammonium Sulphate Precipitation

The ammonium sulphate precipitation has been carried out by addition of varying concentration (10 % to 100 %) of ammonium sulphate under stirring conditions. The precipitated

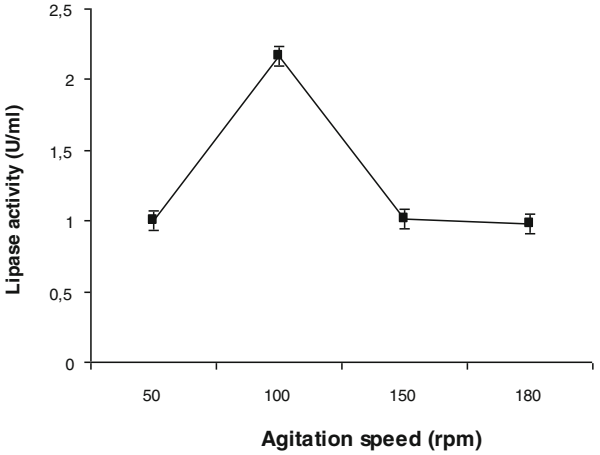

Fig. 6 Selection of agitation speed of media for lipase production. All values are represented as ±SD of three replications. (Experimental conditions: inoculum size, 10 %; incubation period, 3 h; temperature, 35 °C; pH 8.0; carbon source, 12 % soybean oil)

Table 2 Partial purification profile of *S. arleatte* lipase

Step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	% Recovery
Crude	354.00	299.45	1.18	1.00	100
$(NH_4)_2SO_4$ precipitation (60 %)	110	25	4.40	3.72	31.07

protein was collected through centrifugation (6,987×*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 greater. A purification fold of 3.72 and an enzyme yield of 31 % were obtained (Table 2).

Chahinian et al. [30] and Hiol et al. [31] reported the effective recovery of extracellular lipases from *Penicillium cyclopium* and *Rhizopus oryzae* through ammonium sulphate precipitation. Salting out through ammonium sulphate stabilizes the proteins against denaturation, proteolysis and bacterial contamination [32].

Characterization of Lipase

Effect of pH on Lipase Activity

Changes in pH will affect the protein structure and the enzyme activity [33]. 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. There was substantially less activity at pH 4 and pH 5.0. So the enzyme was only active in alkaline pH range. Kumar et al. [34] 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 %). Figure 7 depicts that the enzyme showed stability in the pH range of 7–12.

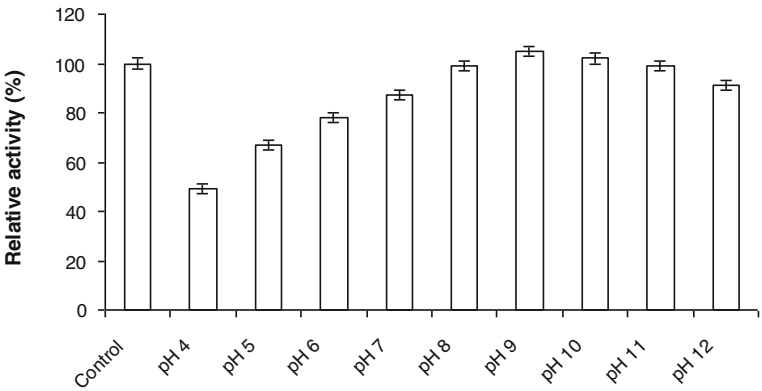

Fig. 7 Effect of pH on *S. arlettae* lipase activity. All values are represented as ±SD of three replications

Effect of Temperature on Lipase Activity

The effect of temperature on lipase activity was analyzed by carrying out assays at different temperatures (30–100 °C). With the rise in temperature the enzyme activity started increasing and reached an optimum at 37 °C (Fig. 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 great 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 enzyme [35]. In contrast to *Staphylococcus similans* lipase, which is inactivated after a few minutes when incubated at 60 °C [36], *S. arlettae* retained 90 % or 60 % of its activity up to 60 min at 55–60 °C, respectively.

Effect of NaCl Concentration on Lipase Activity

The effect of NaCl concentration lipase activity was studied by incubating enzyme with different percentage of 0 to 30 % of NaCl. Figure 9 shows that *S. arlettae* lipase could produce in 0–25 % NaCl, but the best growth was observed in medium without NaCl. This showed that *S. arlettae* should be classified as halotolerant 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 [37].

Effect of Organic Solvents

Exposure of *S. arlettae* to various organic solvents for 60 min showed that this enzyme retained activity in all organic solvents tested. The highest relative activity was achieved at 168.0 %, 143.0 %, and 141.8 % in xylene, benzene, and toulene, respectively (Fig. 10). However, the activities were decreased when the enzymes incubation were extended to 2 h in organic solvents. *Staphylococcus* lipase was very stable when incubated with benzene,

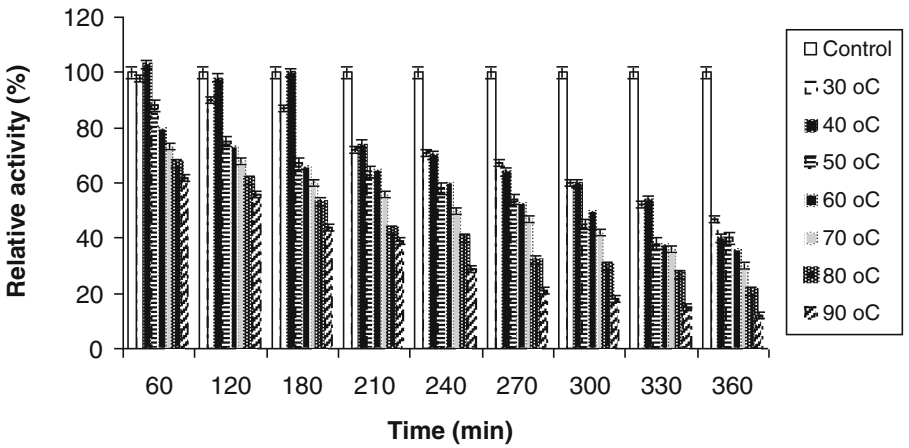

Fig. 8 Effect of temperature on lipase activity. All values are represented as ±SD of three replications

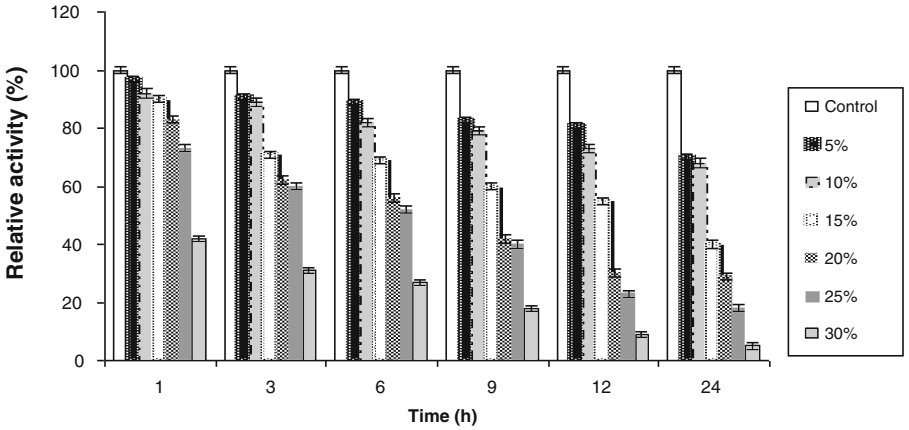

Fig. 9 Effect of different NaCl concentration on lipase stability. All values are represented as ±SD of three replications

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 [38, 39]; hence, activity and stability in organic solvents are considered novel attributes in a lipase.

Effect of Metal Ions on Lipase Activity

To study the effect of various metal ions on lipase activity, the enzyme was incubated with 1 mM of $CaCl_2$, $MnCl_2$, $CoCl_2$, $FeCl_3$, $HgCl_2$, KCl and NaCl for 1 h and enzyme activity was determined following the same standard assay protocol discussed in Section 2.4. Previously, it has been demonstrated that the activity of staphylococcal lipases may depend on the presence of Ca^{2+} ions [36]. It has been reported that the lipases from *P. glumae* [40]

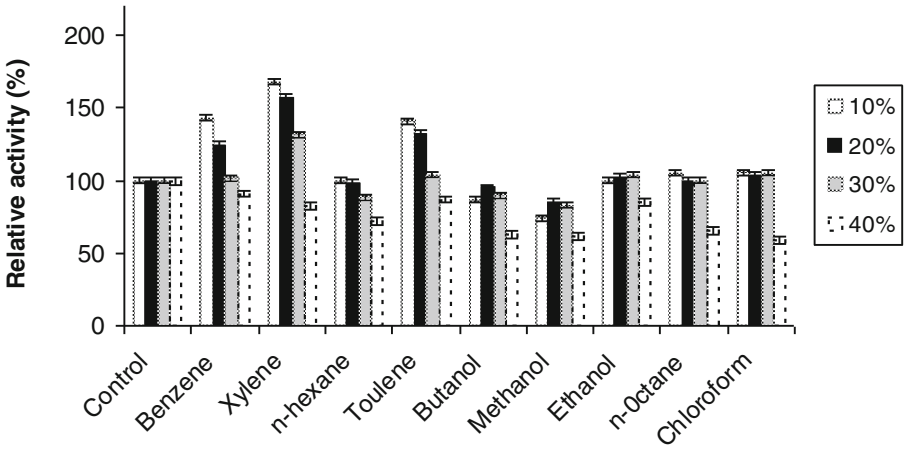

Fig. 10 Stability of lipase in organic solvents. All values are represented as ±SD of three replications

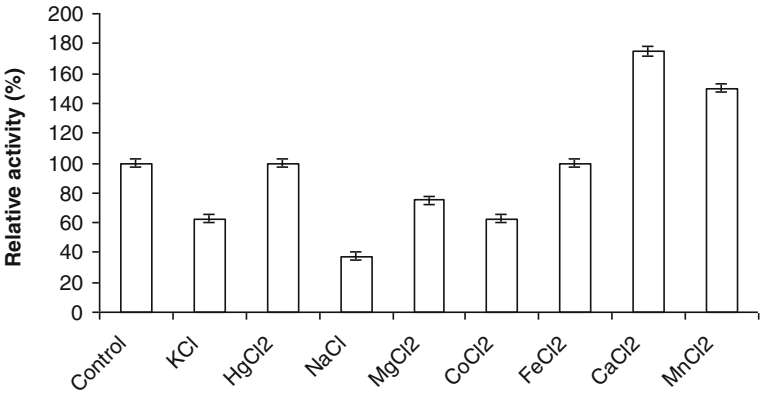

Fig. 11 Effect of different metal ions on lipase activity. All values are represented as ±SD of three replications

and *S. hyicus* [41] 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. [42] 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 presence of 1 mM $CaCl_2$ and $MnCl_2$ the enzyme activity increased. All the other metal ions inhibited the activity of lipase (Fig. 11).

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. 12). The stimulating effect of surfactants on enzymatic hydrolysis has been reported several times [43]. Ebrahimpour et al. [44] and Castro et al. [45] found that lipase activity of *Bacillus* sp. was enhanced in the presence of Triton X-100. The Lip-SBRN2 exhibited a high

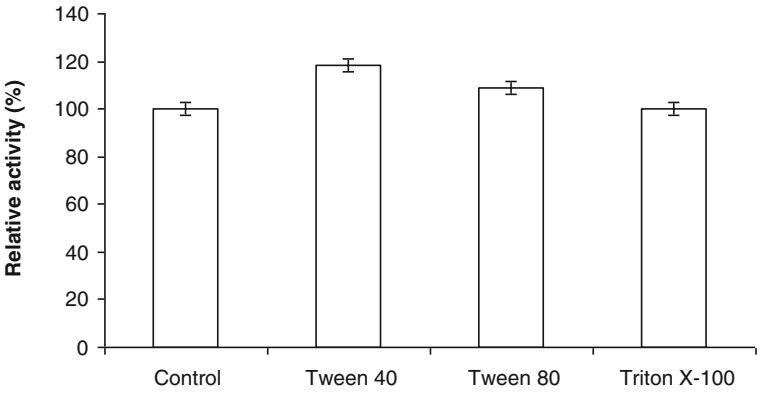

Fig. 12 Effect of surfactants on lipase activity. All values are represented as ±SD of three replications

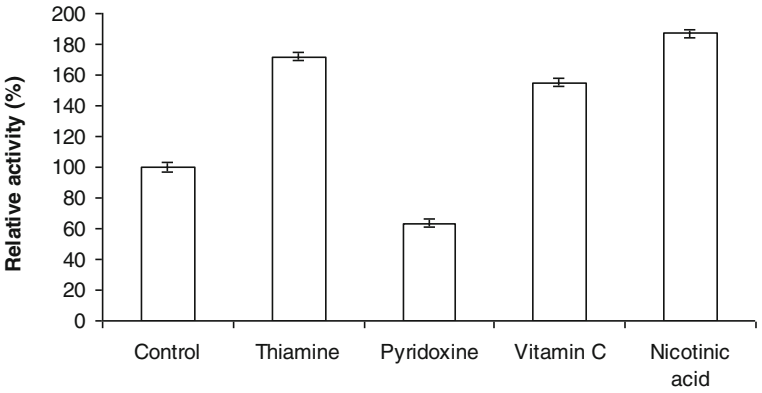

Fig. 13 Effect of vitamins on enzyme relative activity. All values are represented as ±SD of three replications

level of activity in the presence of SDS [46]. It was observed that lipase production was affected in 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. 13) Nicotinic acid stimulated maximum lipase in *Curvulariam pellescens*, *Fusarium equiseti* and *Trichoderma viridae* [47].

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 thiocynate for 1 h showed drastic inhibition in lipase activity. Inhibition of activity with serine inhibitors shows that this lipase belongs to the class of serine hydrolases [48, 49]. 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 thiocynate [50].

The chelators, EDTA and *o*-phenanthrolein were studied for their influence on lipase activity at a concentration of 1 mM. The enzyme activity has been observed to increase in the presence of 1 mM EDTA and lipase activity has been diminished with 1 mM *o*-phenanthroline (Fig. 14). Enhanced and diminished activities in the presence of EDTA and *o*-phenanthroline have been

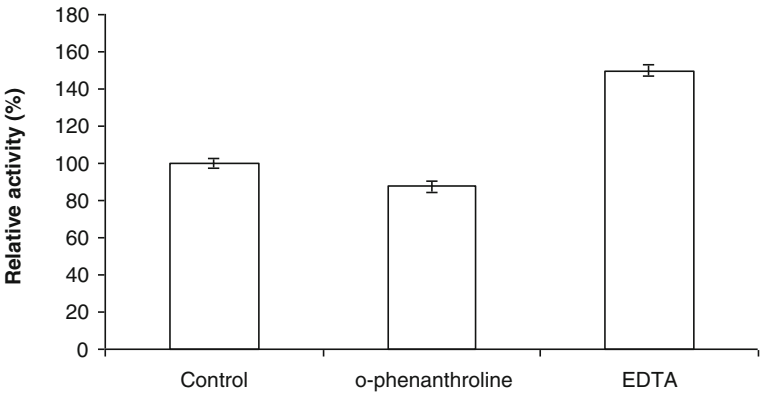

Fig. 14 Effect of chelators on lipase relative activity. All values are represented as ±SD of three replications

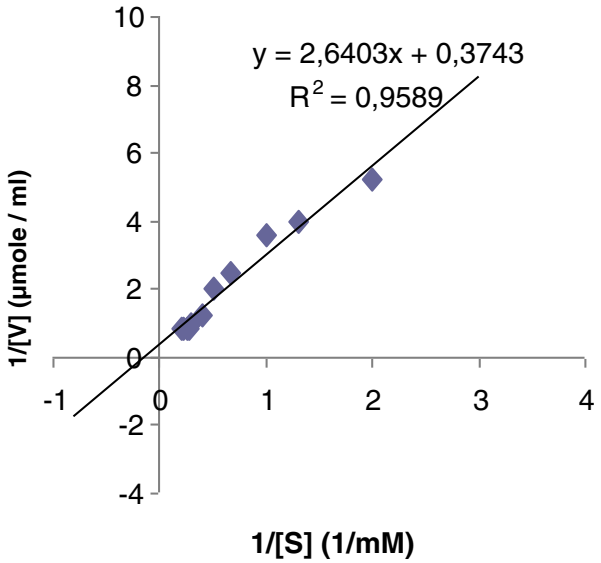

Fig. 15 The Lineweaver–Burk plot of free and *S. arleatte* lipase

attributed to the non-requirement and requirement of cofactor for lipase activity, respectively [48, 50]. The inhibition results with *o*-phenanthrolein have been also reported in case of *Penicillium chrysogenum* [51].

Kinetic Constants of *S. arleatte* Lipase

Basic enzyme kinetics such as K_m and V_{max} are generally 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 the Lineweaver–Burk plot (Fig. 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 *Pseudomonas cepacia* using pNPP as substrate. The kinetic constants of lipase from *S. aureus* have been reported as 14.53 mM (K_m) and 1485 μM/min/mg (V_{max}) [52]. Pencreac'h and Baratti [53] reported K_m and V_{max} values of 12 mM and 30 mmol/min, respectively, for *P. cepacia* lipase using pNPP as a substrate.

Conclusions

The properties of a novel, halo-thermo-solvent-detergent-tolerant lipase by *S. arlettae* showed several advantageous features for industrial applications, which may be helpful for possible applications in the detergent, leather, pharmaceutical, and cosmetic industries. 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.

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