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Full Length Research Paper

Production and characterization of lipase from *Bacillus stearothermophilus*

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The production of lipase by *Bacillus stearothermophilus* was investigated and its properties were evaluated. Different factors such as temperature, pH, carbon source, incubation period and culture volume were studied for improving lipase production. *B. stearothermophilus* was able to produce lipase at a wide range of temperature and pH values. The maximum enzyme activity of 90.569±0.068 U/ml was recorded when the microorganism was grown in a medium containing olive oil as a carbon source and supplied with Tween 80 at a temperature of 45°C and a pH of 8.0 for 24 h of fermentation. The enzyme showed good stability and tolerance for various parameters studied, with residual activity above 50% over a wide range of temperatures. Lipase was stable at pH 8.0 showing 99.64% of residual activity while it lost its stability almost completely at pH 6.0 and 10.0. The enzyme exhibited a moderate stability in organic solvents and seemed to be activated by isopropanol at a concentration of 25 and 100%. The enzyme retained more than 94% of its activity in a buffer system supplied with EDTA and retained over 80% of its activity in a buffer system supplied with Zn^{2+} compared to the metal ions investigated. These results show a great potential for the use of this enzyme in industry and other future studies.

Key words: Lipase, *Bacillus stearothermophilus*, optimization, enzyme characterization.

INTRODUCTION

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are a class of enzymes that catalyze the hydrolysis, esterification and transesterification of long-chain triacylglycerols at an oil-water interface (Meghwanshi et al., 2006; Treichel et al., 2010). These reactions are widely used in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufacturing and production of cosmetics (Hasan et al., 2006; Ma et al., 2006).

In order to be used in industry, lipases need to be thermostable and maintain their activity in organic solvents (Hasan et al., 2006; Nawani and Kaur, 2007). Thermostability is required since many processes use temperatures around 50°C due to the high melting points of the lipids that are used as substrate (Lima et al., 2004). The industrial demand for thermostable lipases stimulates the search for microorganisms that produce such thermostable enzymes. The advantages of running bioprocesses at elevated temperatures (example oil biodegradation), favourable changes in most physical properties of fats at high temperature and increased stability of thermostable lipases to organic solvents determine the increased interest in new thermostable enzymes for new applications (Kambourova et al., 2003). The number of available lipases increased due to multiple achievements in this field and nevertheless, the demand for these biocatalysts has increased especially if they have unique properties like specificity, stability, pH and temperature optima (Treichel et al., 2010; Romdhane et al., 2010).

Microbial enzymes are more useful than enzymes from plants or animals because of the great variety of catalytic activities available, possible high yields, genetic manipulation, continuous supply due to absence of seasonal fluctuations and rapid growth on inexpensive media. They are also more stable than plant and animal enzymes and their production is more convenient and safer (Hasan et al., 2006).

Many microorganisms are capable of producing lipase

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and *Bacillus* sp. is the most widely studied group. *Bacillus* species lipases have been purified and biochemically characterized from many *Bacillus* species such as *Bacillus subtilis*, *Bacillus* pumilus, *Bacillus stearothermophilus, Bacillus thermocatenulatus* and *Bacillus thermoleovorans* and their genes have been cloned and sequenced (Sifour et al., 2010; Kim et al., 2002). *Bacillus* lipases attract attention because they have unique protein sequences and many uncommon biochemical properties (Kim et al., 2002). In contrast, many bacteria other than *Bacillus* sp. have been studied for their lipase production ability (Gupta et al., 2004), among them are *Arthrobacter* sp. (Sharma et al., 2009), *Pseudomonas* sp. (Meghwanshi et al., 2006) and *Staphylococcus* sp. (Horchani et al., 2009). Lipases with new specificities are needed and the engineering of cloned enzymes as well as the isolation of new lipases from natural sources is of increasing potential value (Hasan et al., 2009). The aim of the present investigation was to explore and improve the ability of a newly isolated *B. stearothermophilus* strain isolated from raw petrol sample to produce lipase and study its properties.

MATERIALS AND METHODS

Microorganism and inoculum preparation

Lipase producing *Bacillus* strain was isolated from a raw petrol sample collected from Al-Zarqa city refinery plant (Jordan) and identified as *B. stearothermophilus* according to its physiological characteristics using biochemical procedures (Holt et al., 1994). Backup cultures were maintained on Mueller-Hinton agar slants and were re-cultured every 3 months. For the preparation of the inoculums, *B. stearothermophilus* was inoculated into 50 ml of Mueller-Hinton broth in a 250 ml Erlenmeyer flask and incubated for 8 h at 45°C and 150 rpm (Lima et al., 2004).

Lipase production

About 2% v/v of the seed culture was used to inoculate several 250 ml Erlenmeyer flasks, each containing 150 ml of a medium composed of (g/L): KNO_3, 3.54; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.38; $FeSO_4 \cdot 7H_2O$, 0.01, yeast extract, 5.0 and 1% (v/v) olive oil, adjusted to a pH 8.0 using 1.0 M NaOH and HCl. The flasks were incubated at 45°C for 72 h in a shaker incubator (HumanLab, Korea) at 150 rpm. Growth was followed by measuring the absorbance at λ=550 nm using spectrophotometer (Major Science, USA) and the optical density was converted to colony forming units (CFU). After incubation, the contents of each flask were centrifuged at 6000 rpm for 30 min (Wagtech centrifuge, UK) and the supernatant was used for protein and enzyme analysis.

Optimization experiments

Various parameters like pH, temperature, incubation period and media components were altered to obtain the optimum conditions for the production of lipase by *B. stearothermophilus*. The effect of initial pH on lipase production was studied over pH ranges of 6.5 to 9, while the effect of temperature was determined by incubating the culture over a temperature range of 37 to 60°C. Different carbon sources in the medium were examined to determine their effect on lipase production.

To study the effect of mode of process operation, the production of lipase was carried out in batch and fed-batch process. The experiments were carried out in 500 ml Erlenmeyer flasks each containing 200 ml media. Every 24 h, a 20 ml of fresh media were fed and aliquots of 3 ml culture were withdrawn aseptically for analysis. To study the effect of culture volume, a 5 L bioreactor was used with an initial volume of 3 L culture and after 24 h of fermentation, 50 ml of the fresh media were fed and aliquots of 3 ml culture were periodically sampled for analysis until the experiment was terminated.

Enzyme activity assay

Lipase activity was determined using *p*-nitrophenyl palmitate (pNPP) (Sigma, USA) as substrate (Ertuğrul et al., 2007). The substrate solution was prepared by freshly mixing solution A (30 mg of pNPP in 10 ml of isopropanol) with solution B (0.1 g of gum Arabic and 0.4 ml Triton X-100 in 90 ml of 50 mM Tris–HCl buffer, pH 8) while stirring until all was dissolved. The mixture of 9 ml of substrate solution and 1 ml of enzyme solution was incubated at 60±0.1°C for 15 min and absorbance was measured at λ = 410 nm (Jasco V-530 uv/vis spectrophotometer). The coefficient of extinction (ε) of p-nitrophenol (pNP), under the conditions described, was determined from the absorbance at λ = 410 nm of standard solutions of pNP (0.01 to 0.1 µmol/ml) (ε_{410} = 14.653 L/mol/cm). Suitable controls were made for each experiment. One unit of enzyme activity was expressed as 1 nmol of p-nitrophenol released per minute under the assay conditions. Dissolved protein concentration was determined according to Lowery et al. (1951) method, using bovine serum albumin (BSA) as a standard.

Lipase characterization

In order to study the enzyme stability at various conditions, lipase activity against pNPP was measured using the standard pNPP method. The residual activity was determined and expressed as the percentage of the enzyme activity assayed at pH 8.0 and a temperature of 60°C. To study the effect of temperature on enzyme activity, lipase activity was determined using temperature values between 25 and 70°C. The enzyme thermostability was studied at a temperature range between 25 and 80°C and the residual activity was calculated after an incubation period of 1 hr (Lima et al., 2004). The effect of pH on enzyme activity was evaluated by measuring the enzyme activity at 60°C using various buffers at pH value range between 6.0 to 9.0 (0.05 M of potassium phosphate with pH 6.0, and 7.0; 0.05 M Tris-HCl with pH 8.0 and 9.0). Molar extinction coefficients of pNP at different pH values were used to calculate the activity of the enzyme, and were determined from the absorbance at λ=410 nm of standard solutions of pNP at each pH value. The coefficient of extinction of (pNP) at pH 6.0, 7.0, and 9.0 were ε = 1.936, 7.194 and 7.749 L/mol/cm respectively. The effect of pH on enzyme stability was studied by incubating the enzyme at 30°C for 1h in buffers of pH values ranges from 6.0 to 10.0 (0.1 M potassium phosphate with pH 6.0 and 7.0; 0.1 M Tris-HCl pH 8.0 and 9.0, 0.1 M Glycine-NaOH with pH 10.0).

To study the enzyme stability in organic solvents; 0.1 ml of the enzyme was mixed with 0.9 ml of solvent (Isopropanol, Ethanol or Acetone at different concentrations) for 1 h and the residual activity was measured as explained in the foregoing. Further more, to determine the effect of metal ions and EDTA on lipase stability, the enzyme was assayed as mentioned earlier but in the presence of 5 mM of; EDTA, Ca^{+2}, Cu^{+2}, Mg^{+2}, Mn^{+2} and Zn^{+2} that were obtained by dissolving the relative amounts of $CaCl_2$, $CuCl_2.2H_2O$, $MgCl_2.7H_2O$, $MnCl_2.6H_2O$ and $ZnSO_4.7H_2O$, respectively in 0.05 M Tris-HCl buffer at pH 8.0 (Romdhane et al., 2010).

Table 1. Effect of incubation temperature on lipase activity, protein concentration and growth of *B. stearothermophilus*. Each is represented as mean ± SEM (standard error of the mean).

Temperature	Activity (U/ml)	Protein (µg/ml)	CFU
37	12.170 ± 1.570	707.2 ± 11.2	804±18
45	12.466 ± 1.547ns	884.0 ± 24.4*	733±10**
55	04.072 ± 0.432*	963.2 ± 11.2*	381±17***
60	04.368 ± 0.819*	872.2 ± 48.6*	216±02***

*,**,*** indicate correlations significances at ≤0.05, ≤0.01 and >0.001 and ≤0.001 probability levels, respectively. ns indicates no significant difference is obtained as $p>0.05$. Significance is compared with values acquired at 37 °C.

Statistical analysis

The results were statistically analysed using GraphPad PRISM version 5.02 program. Protein concentration and molar extinction coefficient of pNP were analyzed by linear regression analysis. One-way ANOVA was used for testing the differences between parameters and Tukey's Multiple Comparison Test was used to determine difference between each factor.

RESULTS AND DISCUSSION

Lipase production

Effect of temperature

In order to determine the optimum temperature for growth and lipase production by *B. stearothermophilus*, the isolate was grown in the production medium at various temperatures; 37, 45, 55 and 60°C. It showed the ability to grow at the respective temperatures, especially at 37°C. This is consistent with Gupta et al. (2004) who reported that *Bacillus* sp. has the ability to grow over a wide range of temperatures ranging over 28 to 60°C. The highest level of lipase activity of 12.466 ± 1.547 U/ml was recorded using an incubation temperature of 45°C (Table 1). Though a decrease in the lipase activity was observed at temperatures above 45°C, high content of dissolved protein was noticed. Kim et al. (2000) reported a *B. stearothermophilus* that showed optimum growth at 55°C, but showed almost no activity at the same temperature. On the other hand, Sifour et al. (2010) reported a *B. stearothermophilus* that produced high yields of lipase at 55 and 60°C (Table 1).

Effect of pH

The effect of pH on lipase production was determined by growing *B. stearothermophilus* in the production medium at different pH values (6.50, 7.00, 8.00 and 9.00). The culture medium for *Bacillus* cell growth is usually neutral to slightly alkaline (pH 7.0-8.5) (Gupta et al., 2004). The results show the ability of *B. stearothermophilus* to grow and produce lipase at any investigated pH value being slightly affected by it (Table 2). Berekaa et al., (2009) reported a *B. stearothermophilus* that produced lipase and grew optimally at pH 7.0 and found almost no activity at pH 9.0 and 10.0 compared to the other tested pH

Effect of carbon source on lipase production

The major factor for the expression of lipase activity has always been reported as the carbon source, since lipases are inducible enzymes (Gupta et al., 2004). These enzymes are generally produced in the presence of a lipid such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts and glycerol (Sharma et al., 2009). Different carbon sources were tested for their ability to support lipase production (Table 3). The results indicate that *B. stearothermophilus* was able to utilize the carbon sources for the production of lipase, though maximum growth was obtained using olive oil as substrate (Table 3). This is in agreement with Gupta et al. (2004) where they reported that *Bacillus* lipases have been shown to be induced by olive oil, corn oil, Tween 80, glycerol and glucose. Sekhon et al. (2006) reported that the addition of oils to growth medium especially soybean oil enhanced lipase production and that the incorporation of Tween-80 at various concentrations into the production medium enhanced both growth and enzyme production by *B. megaterium* AKG-1, while Kumar et al. (2011) determined that the addition of 0.5% v/v Tween 80 was necessary to produce more lipase by *B. pumilus* RK31. Lipidic carbon sources seem to be essential for obtaining a high lipase yield (Triechel et al., 2010). Where as Sifour et al. (2010) reported that a *B. stearothermophilus* strain-5 had shown maximum activity when using glycerol, Tween 80 and glucose as a carbon source. *B. stearothermophilus* showed minimal growth when using glucose as a carbon source while showing maximum growth, protein content and lipase activity when supplementing it with olive oil and Tween 80 (Table 3).

Time profile of lipase production

Lipase production in batch culture over 96 h of incubation period was studied (Figure 1). The data show that the

Table 2. Effect of growth medium pH on lipase activity, protein concentration and *B. stearothermophilus* growth. Each is represented as mean ± SEM.

pH	Activity (U/ml)	Protein (µg/ml)	CFU
6.5	7.530 ± 0.614	767.6 ± 05.2	814 ± 162
7.0	7.120 ± 0.069^{ns}	570.2 ± 93.4^{ns}	921 ± 75^{ns}
8.0	8.098 ± 0.364^{ns}	734.8 ± 35.2^{ns}	946 ± 3^{ns}
9.0	5.323 ± 0.910^{ns}	819.6 ± 23.6^{ns}	464 ± 69^{ns}

^{ns} indicates no significant difference is obtained as $p>0.05$. Significance is compared with values acquired at pH 6.5.

Table 3. Effect of carbon source on lipase activity, protein concentration and growth of *B. stearothermophilus*. Each is represented as mean ± SEM.

Carbon source	Activity (U/ml)	Protein (µg/ml)	CFU
Glucose	83.184 ± 12.178ns	769.6 ± 119.2ns	340 ± 9**
Corn oil	78.209 ± 09.188ns	665.1 ± 050.3ns	727 ± 73ns
Olive oil	80.135 ± 09.1820	754.3 ± 043.50	939 ± 28.0
Palm oil	84.564 ± 03.584ns	706.9 ± 040.8ns	498 ± 67*
Sun flower oil	79.559 ± 09.017ns	579.2 ± 041.2ns	724 ± 168ns
Triolein	78.361 ± 09.179ns	694.0 ± 037.7ns	837 ± 103ns
Tween 80+olive oil	82.759 ± 2.093ns	913.2 ± 87.6ns	1056 ± 3ns

** indicates correlation significance at ≤0.01 and >0.001 probability levels. ^{ns} indicates no significant difference is obtained as $p>0.05$. Significance is compared with values acquired for olive oil.

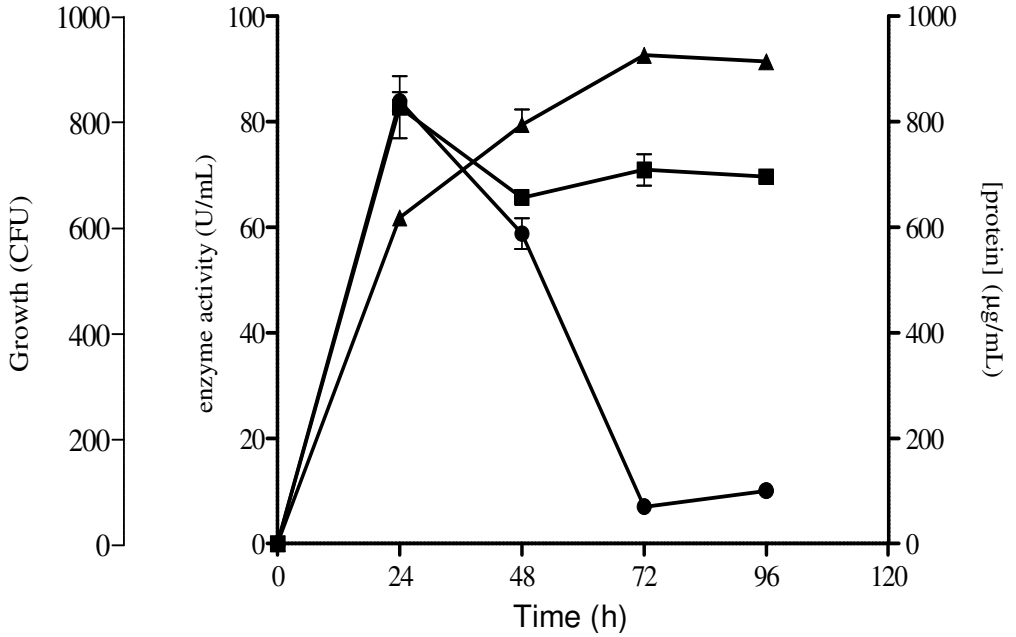

Figure 1. Effect of incubation time on lipase activity ●, protein concentration ■ and growth ▲, during batch cultivation of *B. stearothermophilus*.

maximum lipase activity was obtained after 24 h of incubation, indicating that lipase was necessary for the first stages of growth, while maximum growth was detected after 72 h (Figure 1). Sekhon et al. (2006) reported that the optimum production of extracellular lipase by *B. megaterium* AKG-1 was after 34 h while the highest growth was after 27 h. Berekaa et al. (2009) reported a lipase enzyme by *B. stearothermophilus* that

Table 4. Effect of the mode of operation on lipase activity, protein concentration and growth of *B. stearothermophilus*. Each is represented as mean ± SEM.

Parameter	Time (h)	Activity (U/ml)	Protein (µg/ml)	CFU
Batch	24	90.562 ± 0.0680	931.4 ± 12.20	971 ± 120
	48	83.714 ± 1.729ns	906.8 ± 2.4ns	848 ± 8***
Fed batch	24	70.452 ± 1.479***	0955.4 ± 05.0ns	687 ± 1**
	48	87.809 ± 1.865ns	0978.0 ± 00.8ns	842 ± 10**
	72	83.077 ± 0.045**	1022.4 ± 13.6**	838 ± 3**
	96	83.532 ± 0.864**	0975.2 ± 09.0ns	674 ± 20***

, * indicate correlation significance at (≤0.01 and >0.001) and (≤0.001) probability levels, respectively. ns indicates no significant difference is obtained as $p>0.05$. Significance is compared with values acquired for the first 24hrs of batch operation.

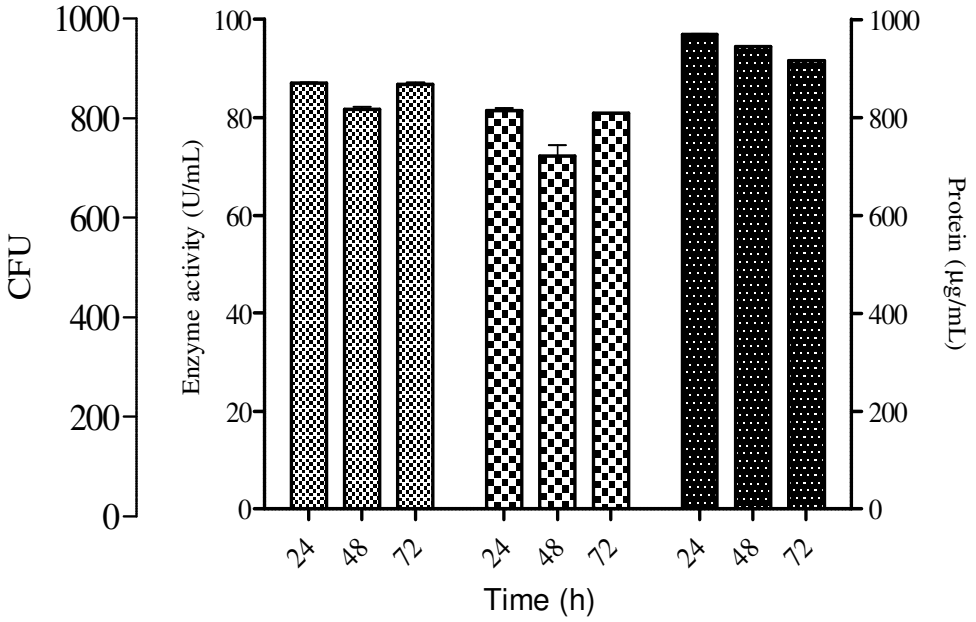

Figure 2. Effect of culture volume on lipase production by *B. stearothermophilus* grown in a 5 L bioreactor at a temperature of 55°C for 72 h. Lipase activity, Protein concentration and Growth.

showed optimum growth after 12 h and optimum lipase enzyme production after 30 h.

Effect of operation mode

When the above optimum conditions were employed in a fed-batch process for lipase production, the enzyme activity profile indicated that the enzyme biosynthesis was affected by the addition of fresh media along with growth. The maximum activity was achieved in batch culture after the first 24 h of fermentation (Table 4).

Effect of culture volume

In order to study the effect of culture volume on enzyme production, a batch process was translated into a bioreactor of a 5 L capacity with a 3 L working volume. Figure 2 shows that the enzyme production profile is in agreement with the previously collected data from shake cultures indicating that scale-up of the process is possible.

Characterization of lipase

Effect of temperature on enzyme activity and stability

Lipase produced by *B. stearothermophilus* showed maximum activity when it was assayed at a temperature of 70°C (Figure 3). Measuring enzyme activity beyond 70°C was not performed because of the spontaneous hydrolysis of pNPP. To determine the thermal stability of

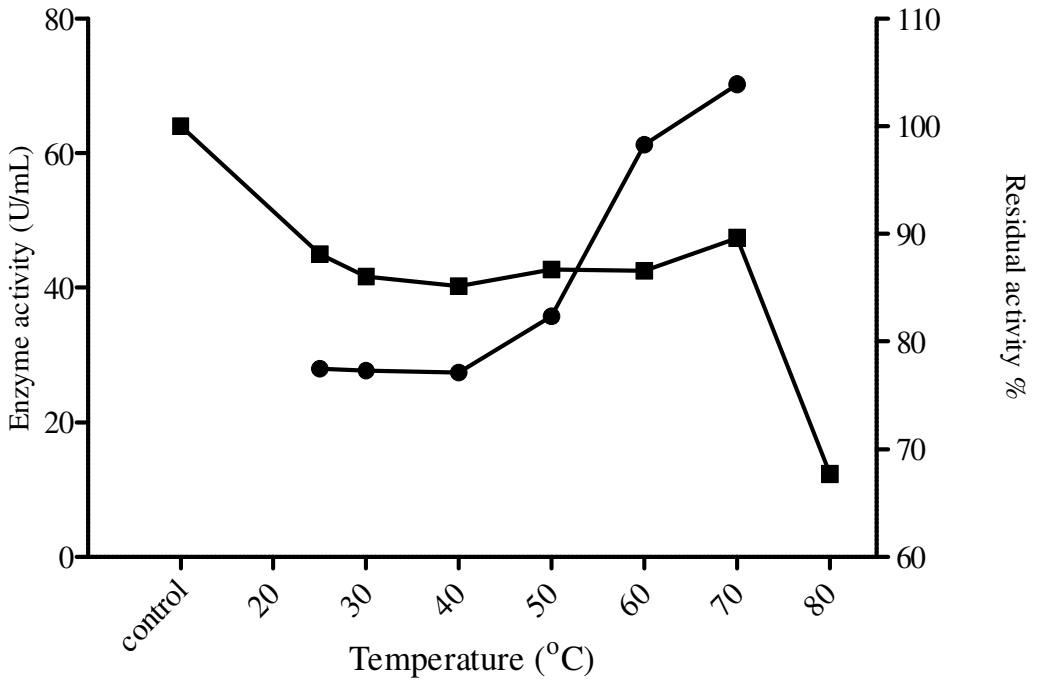

Figure 3. Effect of temperature on enzyme activity (●) and stability (■). For the effect on activity, assay conditions were: 25 to 70°C, 0.05M Tris-HCl buffer pH 8.0. As for the effect of temperature on lipase stability, the assay conditions were: 60°C, 0.05M Tris-HCl buffer pH 8.0. The activities were compared to the activity that was determined in Tris-HCl, pH 8.0, without incubation, and were expressed as percentage.

the lipase, it was incubated in the range from 25 to 80°C (Figure 3), with the residual activity being determined by the standard pNPP method. The results show that the lipase was stable, retaining more than 50% of its activity over the range of temperatures studied. Olusesan et al. (2011) reported a lipase produced by *B. subtilis* NS 8 that had maximum activity at 60°C, with 72% stability when incubated at 50°C for 1 h. Our results are in agreement with Lima et al. (2004) who reported a lipase from *B. megaterium* that showed maximum activity at 55°C and was stable from 30 to 60°C.

Effect of pH on enzyme activity and stability

Figure 4 shows that lipase activity was maximal at pH 6.0 and high in the range of pH 7.0 to 9.0. Nevertheless, the maximum enzyme activity shown at pH 6.0 could be explained by the presence of esterase enzyme that works best at a pH value around 6.0 as explained by Fojan et al. (2000). The lipase remained stable at a pH range of 7.0 to 9.0 after incubation for 1 h at 30°C, with a residual activity remaining above 50% for pH 7.0, 8.0 and 9.0, but showed drastic deactivation at pH 6.0 and 10.0 (16.91 and 5.05%, respectively). These results are in agreement with Kambourova et al. (2003) who reported the production of a lipase by *B. stearothermophilus* MC 7 that had a maximum activity at pH 7.0 to 9.0, but showed inhibition at pH 6.0 and was stable over the range of 6.5 to 11.

Stability in organic solvents

The crude lipase extract was incubated along with various solvents such as isopropanol, ethanol and acetone and its activity was assayed by the standard pNPP assay method. Residual activities were calculated as a percentage of the activity obtained from a control sample incubated in Tris-HCl buffer (Table 5). Notably, the residual activity decreased with solvent concentration, up to 100% (v/v) except for isopropanol where it showed an increase in activity. The lipase maintained its activity and showed high stability when solvent concentration was raised. Kambourova et al. (2003) reported that the lipase produced by *B. stearothermophilus* MC 7 lost more than 50% of its activity in the presence of 50% ethanol and isopropanol. Lima et al. (2004) reported that the lipase from *B. megaterium* showed stability and increase in activity at 25 and 50% isopropanol concentration.

Effect of metal ions on lipase stability

The lipase extract was incubated along EDTA and different metal ions (Ca^{+2}, Cu^{+2}, Mg^{+2}, Mn^{+2}, Zn^{+2}) as

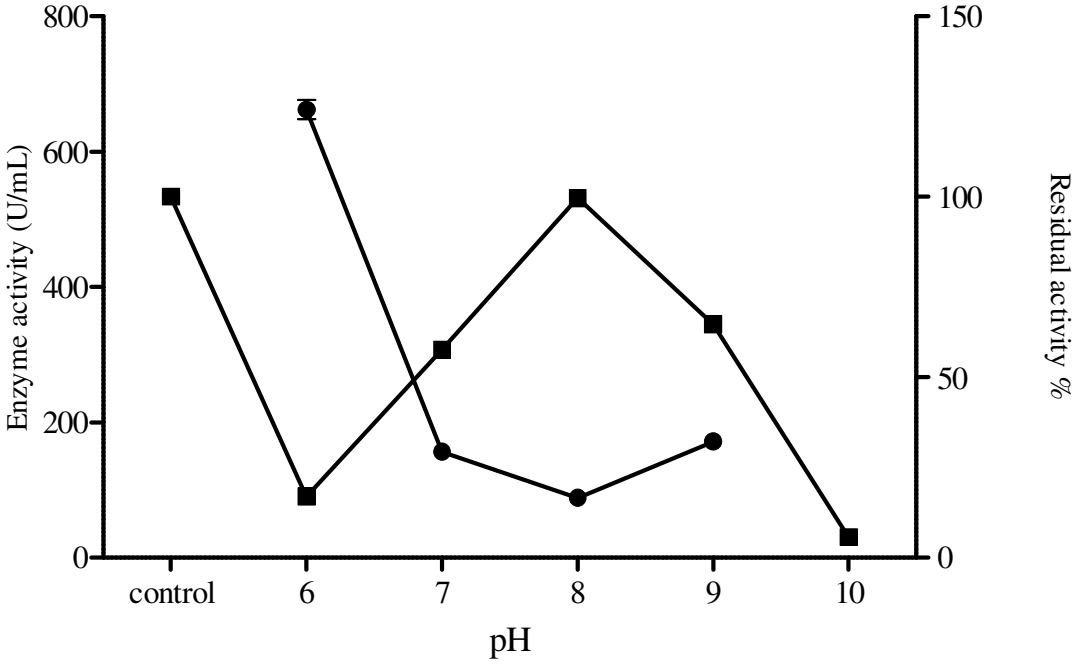

Figure 4. Effect of pH on enzyme activity (●) and stability (■). Activities were measured using the standard assay method with *p*NPP prepared using different buffers. For stability studies residual activities were measured after 1 hr incubation at 30°C, Assay conditions: 60°C, 0.3 mg/ml of pNPP. The activities were compared to the activity that was determined in Tris-HCl, pH 8.0, without incubation, and were expressed as %. Buffers: pH 6.0 and 7.0 phosphate; pH 8.0 Tris-HCl; pH 9.0 Glycine-NaOH, all buffers at 0.05 M.

Table 5. Stability of lipase enzyme obtained from *B. stearothermophilus* after pre-incubation in various solvents. The enzyme was assayed in the presence of solvent for 1 h at 30°C.

Organic solvent	25%		50%		75%		100%	
	Activity (U/ml)	%Residual activity	Activity (U/ml)	%Residual activity	Activity (U/ml)	%Residual activity	Activity (U/ml)	%Residual activity
Control (at 0% organic solvent)	61.785	100.0						
Isopropanol	71.225ns	115.28ns	23.294***	37.70***	47.954*	77.61*	72.795ns	117.82ns
Ethanol	59.146ns	95.73ns	44.360**	71.80**	44.223**	71.58**	23.931***	038.73***
Acetone	61.603ns	99.71ns	22.134***	35.82***	16.265***	26.33***	34.964***	056.59***

*,**,*** indicate correlations significances at ≤0.05, ≤0.01 and >0.001 and ≤0.001 probability levels, respectively. ns indicates no significant difference is obtained as $p>0.05$. Significance is compared with values acquired for the control at 0% organic solvent.

previously mentioned in order to study their effect on its stability. Residual activities were calculated in relation to a control sample incubated in Tris-HCl buffer (Table 6). The lipase showed high stability by retaining over 50% of its activity after 1 h of incubation along with different metal ions. Ma et al. (2006) reported a recombinant lipase from *B. subtilis* with improved activity when incubated in 10 mM Ca^{+2} ions, but showed total inactivation after 30 min of incubation in 10 mM Cu^{+2} ions. Olusesan et al. (2011) showed that the lipase from *B. subtilis* NS 8 was stable in 1.0 mM Ca^{+2}, but was inhibited when treated with 1.0 mM Cu^{+2} ions having 17.1% residual activity after 30 min of incubation. Kambourova et al. (2003) reported that the extracellular lipase produced by *B. stearothermophilus* MC 7 was inhibited by the presence of divalent ions like Cu^{+2} and Zn^{+2} in the reaction mixture, while the enzyme remained completely active in the presence of Ca^{+2} ions.

Conclusion

In this paper, the production of lipase from a newly isolated *B. stearothermophilus* was optimized. The optimum conditions for growth and enzyme production were 45°C, pH 8.0 with olive oil as the carbon source and

Table 6. Effect of metal ions on lipase stability. The activity was measured by incubating the enzyme in Tris-HCl buffer pH 8.0, supplemented with 5mM of the ion for 1 h at 30°C.

Metal ion	Activity (U/ml)	Residual activity (%)
Control	75.297	100.00
EDTA	71.112^{ns}	094.44^{ns}
Ca^{+2}	51.230^{ns}	068.04^{ns}
Cu^{+2}	45.247^{ns}	060.09^{ns}
Mg^{+2}	47.567^{ns}	063.17^{ns}
Mn^{+2}	46.930^{ns}	062.33^{ns}
Zn^{+2}	63.673^{ns}	084.56^{ns}

^{ns} indicates no significant difference is obtained as $p>0.05$. Significance is compared with values acquired for the control.

the addition of Tween 80. The study of the characteristics of this lipase showed its potential for the use in many applications, as it is thermostable, pH tolerant and can withstand a number of metal ions and is stable in high concentrations of organic solvents.

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REFERENCES

Berekaa M, Zaghloul T, Abdel-Fattah Y, Saeed H, Sifour M (2009). Production of a novel glycerol inducible lipase from thermophilic *Geobacillus stearothermophilus* strain-5. World J. Microbiol. Biotechnol. 25: 287-294.

Ertuğrul S, Dönmez G, Takaç S (2007). Isolation of lipase producing *Bacillus* sp. from olive mill wastewater and improving its enzyme activity. J. Hazard. Mater. 149(3): 720-724.

Fojan P, Jonson PH, Petersen MTN, Petersen SB (2000). What distinguishes an esterase from a lipase: a novel structural approach. Biochimie, 82: 1033-1041.

Gupta R, Gupta N, Rathi P (2004). Bacterial lipases: an overview of production, purification and biochemical properties Appl. Microbiol. Biotechnol. 64(6): 763-781.

Hasan F, Shah A, Hameed A (2006). Industrial applications of microbial lipases. Enzyme Microb. Technol. 39: 235-251.

Hasan F, Shah AA, Hameed A (2009). Methods for detection and characterization of lipases: A comprehensive Rev. Biotechnol. Adv. 27(6): 782-798.

Holt JG, Krieg NR, Sneath PHA, Stanley JJ, Williams ST (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilkins: Baltimore.

Horchani H, Mosbah H, Salem N, Gargouri Y, Sayari A (2009). Biochemical and molecular characterisation of a thermoactive, alkaline and detergent stable lipase from a newly isolated Staphylococcus aureus strain. J. Mol. Catal. B. Enzyme 56(4): 237-245.

Kambourova M, Kirilova N, Mandeva R, Derekova A (2003). Purification and properties of thermostable lipase from a thermophilic *Bacillus stearothermophilus* MC J. Mol. Catal. B. Enzyme, 7(22): 307-313.

Kim M, Kim H, Lee J, Park S, Oh T (2000). Thermostable lipase of *Bacillus stearothermophilus*: high level production, purification and calcium dependent thermostability. Biosci. Biotechnol. Biochem. 64(2): 280-286.

Kim HK, Choi HJ, Kim MH, Sohn CB,Oh TK (2002). Expression and characterization of $Ca^{(2+)}$-independent lipase from *Bacillus pumilus* B26. Biochim. Biophys. Acta. 1583: 205-212.

Kumar R, Mahajan S, Kumar A, Singh D (2011). Identification of variables and value optimization for optimum lipase production by *Bacillus pumilus* RK31 using statistical methodology. N. Biotechnol. 28(1): 65-71.

Lima V, Krieger N, Mitchell D, Baratti J, De Filippis I, Fontana J (2004). Evaluation of the potential for use in biocatalysis of a lipase from a wild strain of *Bacillus megaterium*. J. Mol. Catal. B. Enzyme, 31: 53-61.

Lowry O, Rosebrough N, Farr AL, Randall R (1951). Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 265-275.

Ma J, Zhang Z, Wang B, Kong X, Wang Y, Cao S, Feng Y (2006). Overexpression and characterization of a lipase from *Bacillus subtilis*. Protein Exp. Purif. 45: 22-29.

Meghwanshi G, Agarwal L, Dutt K, Saxena R (2006). Characterization of 1-3 regiospecific lipases from new *Pseudomonas* and *Bacillus* isolates. J. Mol. Catal. B. Enzyme, 40: 127-131.

Nawani N, Kaur J (2007). Studies on lipolytic isoenzymes from a thermophilic *Bacillus* sp. Production, purification and biochemical characterization. Enzyme Microb. Technol. 40: 881-887.

Olusesan AT, Azura LK, Forghani B, Abu Bakar F, Mohamed AK, Radu S, Abdul Manap MY, Saari N (2011). Purification, characterization and thermal inactivation kinetics of a non regioselective thermostable lipase from a genotypically identified extremophilic *Bacillus subtilis* NS N. Biotechnol. In press. doi:10.1016/j.nbt.2011.01.002. 8: 2-9

Romdhane I, Fendri A, Gargouri Y, Gargouri A, Belghith H (2010). A novel thermoactive and alkaline lipase from *Talaromyces thermophilus* fungus for use in laundry detergents. Biochem. Eng. J. 53: 112-120.

Sekhon A, Dahiya N, Tewari RP, Hoondal GS (2006). Production of extracellular lipase by *Bacillus megaterium* AKG-1 in submerged fermentation. Indian J. Biotechnol. 5: 179-183.

Sharma A, Bardhan D, Patel R (2009). Optimization of physical parameters for lipase production from *Arthrobacter* sp. BGCC#490. Indian J. Biochem. Biophys. 46(2): 178-83.

Sifour M, Zaghloul TI, Saeed HM, Berekaa MM, Abdel-fattah YR (2010). Enhanced production of lipase by the thermophilic *Geobacillus stearothermophilus* strain-5 using statistical experimental designs. N. Biotechnol. 27(4): 330-336.

Treichel H, Oliveira D, Mazutti M, Luccio M, Oliveira JV (2010). A Review on Microbial Lipases Prod. Food Bioprocess. Technol. 3: 182-196.