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Purification, Characterization and Application of a Cold Active Lipase from Marine *Bacillus cereus* HSS

Sahar WM. Hassan*, Hala H. Abd El Latif, and Ehab A. Beltagy

National Institute of Oceanography and Fisheries (NIOF), Egypt

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Lipases (triacylglycerol acylhydrolases [EC 3.1.1.3]) are water-soluble enzymes. They catalyze the hydrolysis of fats and oils. A cold-active lipase from marine *Bacillus cereus* HSS, isolated from the Mediterranean Sea, Alexandria, Egypt, was purified and characterized. The total purification depending on lipase activity was 438.9 fold purification recording 632 U/mg protein. The molecular weight of the purified lipase was estimated to be 65 kDa using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The optimum substrate concentration, enzyme concentration, pH, and temperature were 1.5 mM, 100 µl, pH 6 and 10℃, respectively. The lipase was tolerant to NaCl concentrations ranging from 1.5 to 4.5%. The lipase was affected by the tested metal ions, and its activity was inhibited by 16% in the presence of 0.05 M SDS. The application of the cold-active lipase for the removal of an oil stain from a white cotton cloth showed that it is a promising biological agent for the treatment of oily wastes and other related applications. To the best of our knowledge, this is the first report of the purification and characterization of a lipase from marine *B. cereus* HSS isolated from the Mediterranean Sea.

Keywords: *Bacillus cereus* HSS, lipase, purification, characterization, oil removal

Introduction

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are considered the milestone hydrolase molecules for biological degradation of triacylglycerols (TAGs) converting this substrate to derivatives of glycerol and fatty acids, in addition to the formation of esters from alcohols and fatty acids, and leading to transesterification processes [1, 2].

The major producer sectors for lipases have been identified from microorganisms so far, providing broad substrate specificity and stability [3, 4]. The genera *Rhizomucor, Candida* and *Rhizopus* among fungi from one side, and *Chromobacterium* and *Pseudomonas* among bacteria from the other side are the common public producers of commercial lipolytic enzymes [5–7]. Also, it was mentioned that lipases produced by *Bacillus* spp., for instance *Bacillus alcalophilus* (*B. alcalophilus*), *B. licheniformis, B. subtilis* and *B. pumilus* are the remarkable producers of lipase exhibiting significant biotechnological applications such as bioremediation of contaminated soil and water, detergents, paper, food additives and synthesis of fine chemicals [8].

Marine microbes can degrade organic matter using different specific pathways, including extracellular enzymes, to yield low molecular masses of enzyme targets. It is a matter of challenging that marine microbes produce extracellular enzymes under restricted laboratory conditions neglecting all possible unseen natural factors. Last decades, psychrophilic lipases paid a great attention [9]. The features of cold-active hydrolase molecules such as lipolytic molecules provide a wide angle of valuable application in food, detergent and textile industries, in addition to wastewater treatment and

***Corresponding author**
Tel.: +0201200285519
E-mail: saharwefky@yahoo.com

bioremediation of pollutants [10]. Cold active lipases hydrolyze fats and are a member of the wider group of lipolytic enzymes, which perform lysis processes. Cold active lipases have developed certain structure that affords both good thermal resistant range and great specific activity at down degree level of temperatures [11]. Such features have opened the chance for giving a potential of applying in different industrial applications such as pharmaceutical preparations, medical, synthesis of critical chemicals, detergent additives, food processing, environmental bioremediation and leather processing [12–14]. Up till now, many researches have concentrated on lipase production from thermophiles [15, 16], although a little attention has been paid to the possible yield maximization of cold-active lipases from the microorganisms in cold environment.

Purification of lipases has a great commercial importance especially those produced from microbial sources, where purification of different lipases obtained from fungi, bacteria, animal and plants have already been occurred [17]. For purpose of lipases purification, different approaches are in usage. As was stated, ammonium sulfate precipitation and DEAE-Sepharose column chromatography can be used for lipase purification [18].

The current study sets out the production of cold active lipase from the marine psychotolerant bacterium *Bacillus cereus* HSS and focuses on purification, characterization and application of the purified enzyme. According to our information, it is the first study on investigating the purification and characterization of cold adapted lipolytic enzyme from the marine *Bacillus cereus*.

Materials and Methods

Strain and chemicals

The psychrotolerant marine *Bacillus cere*us HSS, that was earlier isolated, purified and identified as a marine isolate from Mediterranean Sea, was handled in the current research [1]. Sephadex G-100 and CM-Cellulose (CMC) were obtained from Sigma (Sigma-Aldrich, USA). Fine chemicals with upper grade were applied.

Yield harvest of lipase

Over night culture of *Bacillus cere*us HSS was used as the starter for production of lipase. One milliliter was taken to inoculate 50 ml of the prepared mineral broth medium contained (g/l): sucrose, 0.5; $MgSO_4 \cdot 7H_2O$, 0.05; K_2HPO_4, 0.01; $NaNO_3$, 0.03; $FeSO_4 \cdot 7H_2O$, 0.001; KCl, 0.05 and pH 7. The inoculated medium was cultivated at 10℃ under shake condition for 24 h. Subsequently, centrifugation at 10000 g for twenty minutes was done to obtain cell free supernatant ready for lipase activity detection [19].

Lipase assay

Titrimetric method was applied for estimation of lipolytic activity according to the protocol used by Hassan *et al.* [1] using oil extracted from cotton-seed as a substrate. Briefly, emulsion of mixing Arabic gum prepared in 0.05 mM Tris buffer, pH 7 with cotton-seed oil was set. The lipolytic activity was measured by adding specific volume of enzyme sample (100 µl) to the prepared emulsion and keeping for half an hour in adjusted low temperature (10℃). Afterwards, the reaction was stopped by adding aceton:ethanol with ratio (1:1). The produced fatty acids resulted from degraded oil was titrated against 0.05 N NaOH providing phenolphthalein as an indicator. One unit of lipolytic activity was defined as the required amount of enzyme to liberate 1 µmole of fatty acids from triglyceride per minute.

Protein determination

Protein content measuring as mg/ml was estimated owing to the method of [20]. In steps, 200 µl of sample was added to one ml of Lowry reagent and left for 10 min afterwards, 100 µl of folin-ciocalteau was added and the total volume of 1.3 ml reaction mixture was kept for 20 min or more to develop a color with intensity measured by spectrophotometer at 750 nm.

Partial purification of lipase

The purification phases of the extracellular lipase were carried out at 4℃ using the collected supernatant after high speed spinning at 6,000 rpm for 20 min. In brief, the supernatant was treated with acetone, ethanol and ammonium sulphate crystals giving saturation (30, 50, 70 and 90%) conjugated with uninterrupted stirring for protein precipitation. The precipitate was dissolved using phosphate buffer (0.05 M; pH 6). Dialysis of resulted saturated fractions was performed in phosphate buffer (0.05 M; pH 6) for 24 h with several buffer

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exchanges, followed by lipolytic activity assay.

Ion exchange chromatography

The most active acetone fraction resulted after partial purification of lipase was exposed to cation exchange chromatography using CMC column (2 × 10 cm) after equilibration with phosphate buffer (0.05 M; pH 6). At the beginning, rinsing the column several times with NaCl dissolved in phosphate buffer 0.05 M, pH 6, followed by stepwise grade elution using phosphate buffer 0.05 M, pH 6 with NaCl (0–1 M) and applying a flow rate 1 ml/min. Subsequently, collection was done as 3 ml/fraction for further analysis.

Gel filtration chromatography

Pooled fractions with the highest peak of lipolytic activity resulting from cation exchange column were subjected for further purification using Sephadex G-100 column, after pre-equilibration with 0.05 M phosphate (pH 6). This buffer was also used for elution of protein at flow rate 0.5 ml/min. Assay of protein and lipase activity was carried out for all the pooled fractions (2 ml/fraction).

Characterization of lipase

Molecular mass determination. As described [21], SDS-PAGE (8%) was applied for resolving the lipase molecular mass.

Influence of diverse substrate concentrations. Influence of diverse substrate concentrations (0.1–4 mM) in the reaction mixture was implemented at pH 6.0 and 10℃ for twenty minutes. Lipase activity was estimated at each concentration.

Estimation of kinetic constants. Kinetic parameters were estimated using Line weaver-Burk method [22] following Michaelis-Menten equation outcome to estimate maximal velocity (V_{max}) and Menten constant (K_m). The applied equation was as follow:

$$V1 = V_{max}\,[S] / K_m + [S]$$

Where the reaction velocity is expressed as V1, the maximal velocity is V_{max}, the substrate concentration is [S] and concentration of substrate at half-maximal velocity is K_m.

Impact of varied lipase concentration. Impact of varied concentrations of lipase enzyme ranging from 23.4 to 117 μg, which representing as 50–250 μl/reaction mixture (v / v), on the purified lipase activity was tested. The test was carried out at 10℃ and pH 6.0 for twenty minutes. Lipase activity was estimated at each concentration.

Acidity and alkalinity effects on lipase activity. pH levels (4–10) were tested against lipase activity using different buffer preparations as follow; 0.05 M of sodium carbonate buffer (9.2–10.7), 0.05 M of phosphate buffer (5.6–8.0) and 0.05 M of acetate buffer (3.6–5.4).

Thermal effect on lipase activity and stability. Varied temperature degrees (5℃ to 70℃) were studied for their effect on the activity and stability of the purified lipolytic protein for 15, 30 and 60 min. Remaining activity was recorded at each case under standard settings.

Influence of salinity on lipolytic activity. The impact of varied NaCl concentrations (1.5–4.5%) on the performance of purified lipolytic molecule was estimated for each NaCl concentration.

Influence of surfactants, heavy metals and enzyme blockers on lipase activity. The effect of some heavy metals, activators, enzyme blockers and surfactants (Mn^{2+}, Ni^{2+}, Na^{+}, Fe^{2+}, Cu^{2+}, Cd^{2+}, urea, SDS and EDTA on lipase activity were tested by mixing each solution at (0.05, 0.005 M) concentration separately with lipase for 2 h, then the residual activity were documented and compared to control one.

Application of the purified lipase in removal of oily waste from clothes. The application of lipase in removing oily stains from a fabric was carried out according to the following described steps with minor modification of [23]. A spotless and dirt-free piece of white cotton cloth was dripping in cottonseed oil with 0.1% Congo red dye for 15 min, then drying was done in hot air oven for 5 min at 80℃. The dried piece was divided to equal sizes of 4 × 4 cm^2 and put with the purified lipase at 10℃ for 30 min. After incubation, the tested part was washed

with tap water without scrubbing for 2 min, then dried in open air. The same method was used for the control (lacking exposure to lipase).

Results

Precipitation of crude enzyme

Fractional precipitation with acetone, ethanol and ammonium sulphate (30, 50, 70, 90%) was carried out as the first step of lipase purification. Lipolytic activity and protein assay were measured for the concentrated fractions. According to the obtained results shown in Table 1, the precipitate fraction obtained by 70% acetone exhibited the highest value of lipase activity (220 U/ml) as well as the specific activity (2.17 U/mg). After precipitation with 70% acetone, an increased specific activity from 1.44 to 2.17 U/mg protein with 1.5 fold purification and approximately 38% loss of lipase activity were observed.

Purification of the enzyme

For purification of lipase, multistep plan is assumed to exclude the undesirable proteins and to keep the activity. Upon the partial purification with 70% acetone, the partially purified lipase was applied to cation exchange chromatography. The results imposed the profile of lipase activity, and the eluted protein from the column are shown in Fig. 1 giving the presence of 29 pooled fraction with the highest activity peak (330 U/ml) for the fraction number 14.

Fractions exhibiting the highest activity (9–15) from the Carboxymethy Cellulose (CMC) column were gathered and further purified using Sephadex G-100 column (Fig. 2). After two columns processing, the specific activity of the purified lipase was increased by 438.9-fold purification recording 632 U/mg protein. A summary of lipase purification from *B. cereus* HSS is presented in Table 2.

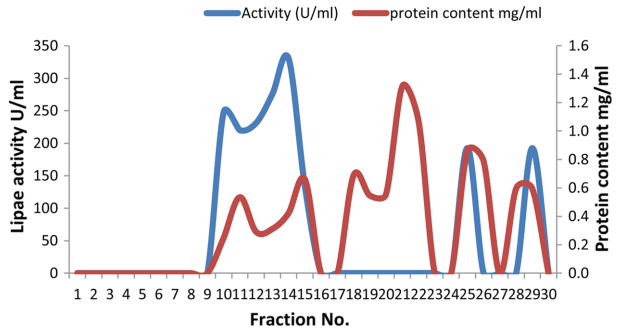

Fig. 1. Cation-exchange chromatography on CMC of the semi purified lipase components giving 29 pooled fractions and showing three active lipase peaks (one major with the highest lipase activity, and two minors), and four protein peaks, where collected fractions of major peak (9-15) was further purified by exposing to the second column.

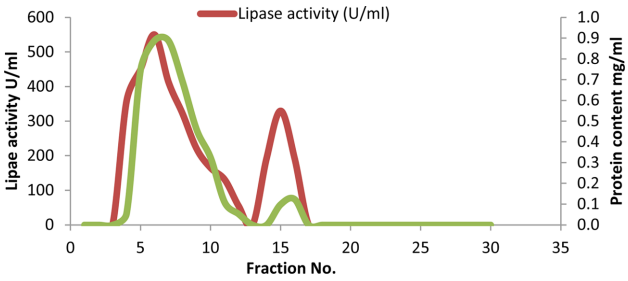

Fig. 2. Gel filtration on sephadex-G100 of the major active lipase components obtained from *B. cereus* HSS giving two active lipase peaks (one major from fraction no. 4 to 12 and one minor peak from fraction no. 14 to 16) concomitant with similar fractions pattern of two protein peaks (one major and one minor).

Characterization of purified lipase produced by *B. cereus* HSS

Molecular mass determination. Standard ladder providing 10 to 170 kDa were applied for determining the molecular mass of the lipolytic protein. The pattern of the purified lipase on SDS-PAGE depicted in Fig. 3 showed one protein band with approximately 65 kDa.

Table 1. Fractional precipitation of lipase using different solvents.

	Acetone		Ethanol		Ammonium sulphate	
	Protein (mg/ml)	Activity (U/ml)	Protein (mg/ml)	Activity (U/ml)	Protein (mg/ml)	Activity (U/ml)
30%	58.70	126.5	93.04	99	68.7	55
50%	148.26	148.5	205.22	55	215.65	82.5
70%	101.30	220	114.35	137.5	84.78	110
90%	77.83	82.5	160.43	93.5	75.22	71.5

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Table 2. A summary of lipase purification from *B. cereus* HSS.

Step	Protein content (mg/ml)	Lipase activity (U/ml)	Specific activity (U/mg)	Specific activity purification fold
Cell free medium	402.1	579.4	1.44	1
Acetone precipitation (70%)	101.3	220	2.17	1.5
Cation exchange chromatography	0.405	239.583	591.56	410.8
Sephadex G-100	0.468	295.78	632	438.9

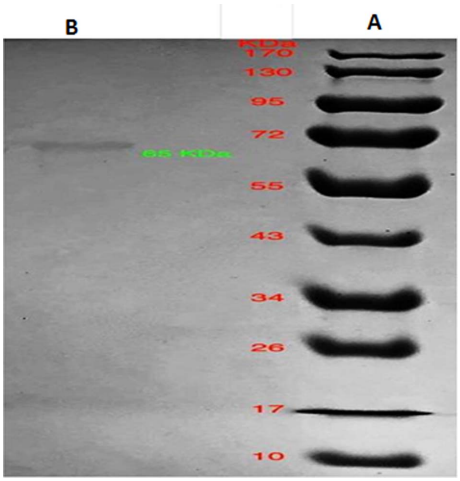

Fig. 3. SDS/PAGE (8%) of the purified lipase (A) molecular weight marker proteins (Page Ruler prestained protein ladder (10-170 kDa), (B) molecular weight of purified lipase from *B. cereus* HSS (65 kDa) stained with Coomassie Brilliant Blue R-250.

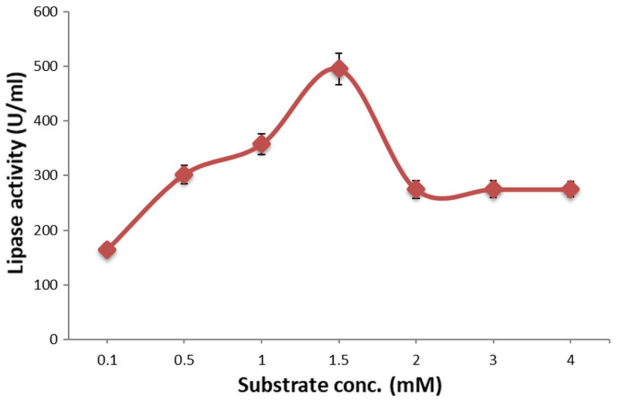

Fig. 4. Effect of different substrate concentration of cotton seed oil ranging from 0.1 to 4 mM on lipase activity produced by *Bacillus cereus* HSS showing the highest activity with 1.5 mM cotton seed oil concentration.

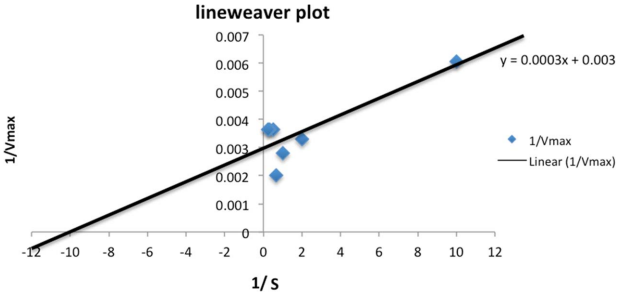

Fig. 5. Lineweaver plot for V_{max} and K_m estimation values, where V_{max} and K_m were 0.623 mM and 3.33×10^3 U/ml, respectively.

Influence of diverse substrate concentration. The influence of varied concentrations (0.1 to 4 mM) of cotton-seed oil on the purified lipid hydrolase activity was noticed. As depicted in Fig. 4, the more gradual substrate concentrations (0.1 to 4 mM), the more increase in the lipolytic activity reaching the highest activity (495 U/ml) at 1.5 mM. However, the activity decreased from 2 mM to 4 mM reaching 275 U/ml.

Lineweaver-Burk plotting was used to evaluate V_{max} and K_m values of lipase. The plot was linear, which pointed to that hydrolysis of cotton-seed oil by lipase followed Michaelis-Menten kinetic with K_m of 0.623 mM toward the substrate and V_{max} of 3.33×10^3 U/ml (Fig. 5).

Impact of varied lipase concentration. Range of lipase concentration by volume between 50 and 250 µl equivalent to the range between 23.4 to 117 µg was tested for lipase activity. The giving results (Fig. 6) showed that the more concentration was applied, the more activity was obtained with approximately a linear pattern. The concentration of 46.8 µg equivalent to 100 µl giving activity 275 U/ml was selected as an average value for forthcoming characterization.

Acidity and alkalinity effects on lipase activity. Different values of pH (4–10) were tested for their effect on lipase

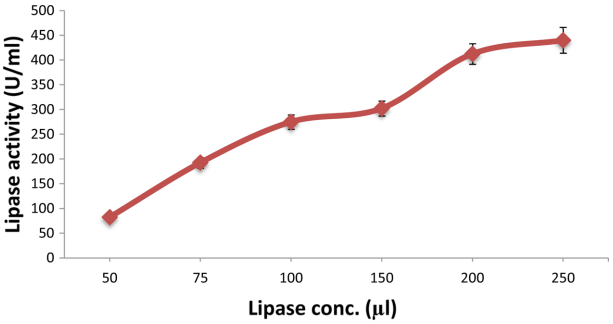

Fig. 6. Effect of different lipase concentration ranging from 23.4 to 117 µg on lipase activity showing a direct correlation between both the concentration and the activity.

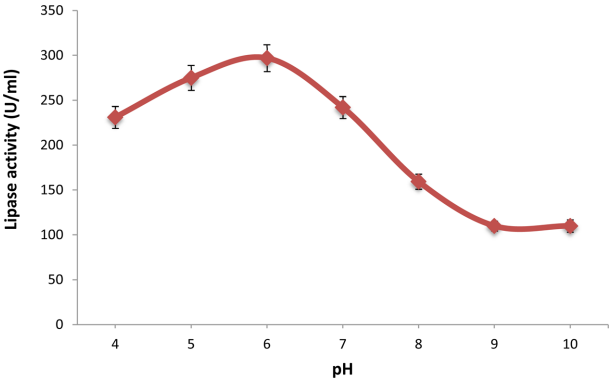

Fig. 7. Effect of different pH (4-10) on lipase activity showing the highest lipase activity at pH 6 (297 U/ml).

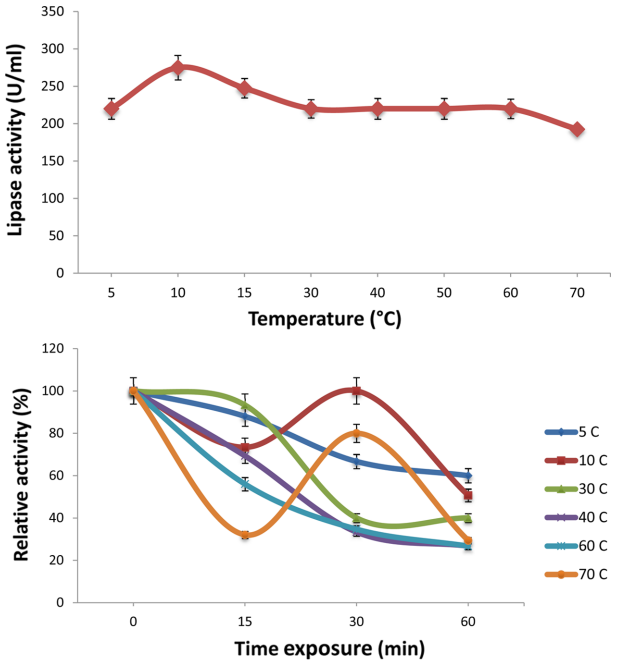

Fig. 8. (A) Effect of different temperature (5-70℃) on lipase activity showing the highest lipase activity at 10℃ (275 U/ml), (B) Thermal stability of the purified lipase while exposing for different temperatures (5-70℃) for different intervals (15, 30, 60 min) showing thermal stability at low temperatures (5 and 10℃) and thermal sensitivity towards higher degrees (30, 40, 60 and 70℃) with direct correlation with time intervals.

activity and stability. The data in Fig. 7 displayed that increasing of original pH of the reaction initiated an elevation in lipase activity up to pH 6 recording the highest activity (297 U/ml), followed by a decrease in the activity recording the lowest activity (110 U/ml) at pH 9 and pH 10 indicating the significant dependency of activity on pH.

Thermal effect on lipase activity and stability. The Influence of temperature on lipase activity was studied at varied temperatures ranged from 5–70℃. As observed in Fig. 8A, the purified lipase exhibited relatively higher activity at low temperature (5–15℃) showing the highest activity (275 U/ml) at 10℃, while almost constant activity (220 U/ml) was observed in the temperature range 30–60℃, followed by smooth decline in the activity at 70℃, which reflects the resistibility nature of the cold lipase enzyme. Thermostability of the purified lipolytic enzyme was detected at different temperature between 5 to 70℃ for exposure time 15, 30 and 60 min. Lipase activity and remaining activity were separately determined each time. As shown in Fig. 8B, the enzyme was adapted giving proper activity in the tested range 5–30℃ keeping over 80% of the original activity after 15 min of incubation and more stability was observed at 10℃ retaining approximately 95% activity after 30 min of incubation and keeping almost 50% of the activity after 1h, while the activity was dropped to 26.67 and 29.33% at temperature ranged from 40–70℃ after 1 h.

Influence of salinity on lipolytic activity. The present study screened different NaCl concentrations ranging from (1.5–4.5%) for their effect on the purified lipid hydrolase enzyme. As shown in Fig. 9, the highest lipase activity (280.5 U/ml) was obtained at 2% NaCl, however, lipase was still active at higher NaCl concentrations reaching 110 U/ml at 4.5% NaCl.

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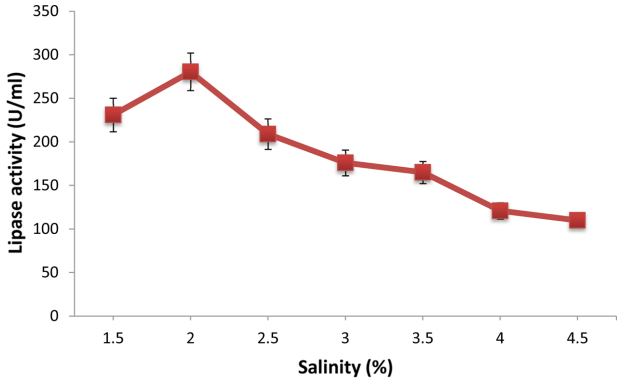

Fig. 9. Effect of different salinity (1.5-4.5%) on lipase activity showing the highest lipase activity at 2% (280.5 U/ml).

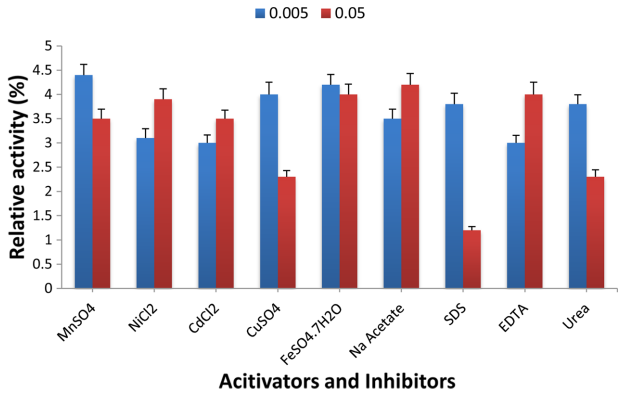

Fig. 10. Effect of different activators and inhibitors concentrations (0.05 M and 0.005 M) on lipase activity showing the relative lipase activity upon each concentration for each factor.

Influence of surfactants, heavy metals and enzyme inhibitors on lipase activity. Influence of some activators and inhibitors on activity of lipase was tested using 0.05 and 0.005 M of each substance. As shown in Fig. 10, generally, the lipase protein expressed a different degree of sensitivity towards all the tested substances and their concentrations showing different degree of inhibition compared to the control (without any addition). The most effective one was the SDS substance at concentration 0.05 M, which brought about 16% of original lipase activity.

Application of the purified lipase in removal of oily dirt. The prospective of the purified lipase for removing the oily dirt was evaluated in the current study. As shown in Fig. 11A, the oily spot was detached from the white cotton cloth after incubating the cloth with 100 µl of purified lipase in the center of stain (cotton-seed oil with Congo red dye) for 30 min comparing to the control (the oily stain without addition of lipase, Fig. 11B), which indicates the effectiveness of the purified lipase for removal of oil dirt located on the fiber surfaces.

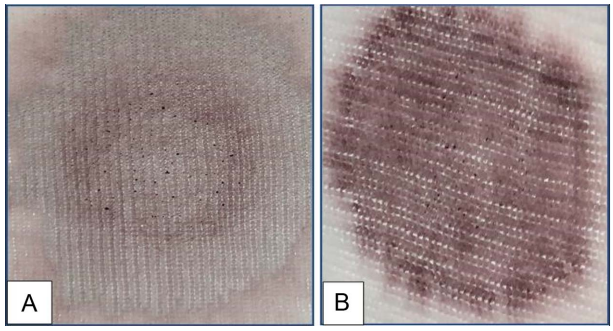

Fig. 11. Removal of oily stains from cotton cloth pieces (4 × 4 cm); (A) Treated by the purified lipase (46.8 µg/100 ul) of *B. cereus* HSS for 30 min, (B) control (without treatment).

Discussion

Scientists and industrialists are taking intense interest in lipases due to their applications in different economic industries such as food, cosmetics chemical industries and pharmaceuticals. Subsequently, certain grade of purity is essential [17].

Purification of lipase was carried out in a sequential manner to obtain a protein of concern and to remove undesired one. The first step was precipitation using acetone, ethanol and ammonium sulphate showing the highest efficiency for acetone (70%) recording the highest lipase activity and specific activity. A previous study recommended the use of acetone with percentages 0–40% [24] for precipitation of lipase produced by *Pseudoxanthomonas* sp., where it exhibited the highest specific activity comparing to the other used fractions.

The current study realized remarkable more purification fold comparing to other previous studies. In support is a study by Heini *et al.* [25] who obtained a purification fold of 385. In a parallel research, the purification of cold active lipase from *Oceanobacillus* strain PT-11 yielded 14.80% extra activity showing specific activity of 65.55 U/mg protein and overall increase in the specific activity with 2.33 fold using Q-Sepharose FF and SP-

Sepharose FF chromatograph [26]. More purification fold of 6.63 with 31.73 U/mg specific activity was obtained after using Diethylaminoethyl A-50 and Sephadex G-100 for lipase purification from *Aspergillus costaricaensis* CBS115574 [27]. The Precipitation of lipase produced by *Bacillus methylotrophicus* PS3 using ammonium sulfate was subjected to Sephadex G-100 column yielding 24.10% and 2.90 fold purification, which was similar finding of Sharma *et al.* [28]. The most likely explanation for the supreme value of specific activity in our study may refer to the observed low value of purified protein that might be happened due to many factors, one of which was the purification steps where ultra-membrane filter (0.22 µm diameter) was used prior to each loading step to the columns. This filtration screened a noticeable amount of greasy particles with high potential of adhesive proteins where the measure of protein content was dropped from 101.3 to 0.405 mg/ml. The other factor could be the purification itself that purify only one single lipase that had the highest activity from many other possible ones. Moreover, a low yield of lipase may refer to striving in elimination of the rich content of lipopolysaccharide existing and coupled with hydrolysis of lipid [29].

After purification the SDS-PAGE analysis showed that the molecular mass of purified lipid hydrolase obtained from *B.cereus* HSS was 65 kDa. The molecular mass was close to that recorded for *Bacillus* sp. THL027 lipase (69 kDa) as was previously reported [30], while was different from that reported in other studies concerning lipases from *B. methylotrophicus* PS3 (31.40 kDa) [28] and *B. licheniformis* NCU CS-5 (28 kDa) [31].

V_{max} and K_m values of lipase were estimated according to Lineweaver and Burk [22] manner. Different values of V_{max} and K_m (5.24×10^5 µM and 16.58 mM) were reported for lipase produced by the psychrophilic *Yersinia enterocolitica* strain KM1 [12].

In the present study the lipase produced by *B. cereus* HSS had maximum activity at pH 6. Similarly, Lanka and Latha [32] have reported that the optimum pH of the cold active lipase was at the acidic range and the enzyme had greater stability in the pH ranged from 4 to 7. In a parallel study [33], the optimum pH for the cold active lipase purified from Antarctic ice bacteria *Pseudoalteromonas* sp. NJ 70 was at pH 7. Also, Salwoom *et al.* [9] have reported that pH 7 was the optimum for lipase isolated from the psychrophilic bacterial strain *Pseudomonas* sp. LSK25 obtained from Signyisland, Antrarctica. The effect of pH may be correlated to changes in charges of amino acids side chains of the enzyme protein, leading to new arrangements of peptide chain in the active site of the enzyme [34, 35].

Bacterial lipases are stable at broad range of temperatures [17]. The optimum temperature for cold-adaptive lipase activity was found to be 10℃. The same observation was indicated in previous studies [9, 36, 37]. However, other authors [38] have reported that the highest activity of lipase was at 15℃ with a drastic decline in the activity above 30℃. Conversely, higher activity was detected at higher temperature for lipases produced by *Pseudomonas* and *Pseudoalteromonas* [33, 36]. In addition, it was mentioned that cold active lipase produced by *Pseudomonas* was stable in a range of 5–40℃ with more resistibility and retained over 80% of its activity after 1 h of incubation, followed by gradual decrease at 50℃ retaining only 35% of the original activity in 10 min and below 10% after 30 min [38].

Screening the impact of varied NaCl concentrations (%) on lipolytic activity was studied showing the highest activity at 2% NaCl. Higher concentrations were needed for more lipase activity, where Musa *et al.* [39] showed that the highest lipase activity from *Marinobacter litoralis* SW-45 was at 12% (w/v) NaCl, while the maximum lipase activity from *Chromohalobacter jabonicus* BK-AB18 was observed at 8% NaCl as was stated [40].

Among the tested activators, inhibitors and SDS, the most effective one on lipase activity was SDS substance at concentration 0.05 M, which brought about 16% of original lipase activity. Less effect of SDS was detected in a previous study [12] giving 60% inhibition of lipase activity. Relatedly, negative charges could be the reason for lipase activity reduction or inhibition, where the electrostatic repulsion between substrate and the enzyme active site could be occurred. This hypothesis was supported by other previous studies [9, 11] that have reported that the addition of transition metals such as Ni, Na, K led to reduction of lipase activity. Also lipase was strongly inhibited by Zn, Cu, SDS, EDTA due to effect on the solubility, changing the enzyme conformation, catalytic properties of the enzyme in addition to behavior of the substrate in the reaction buffer as was stated earlier [41].

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As an applicable part, the current study investigated and approved the capability of the purified lipase for removal of oily stains. In a harmony, previous studies [42–44] reported the same result and indicated that the oil exclusion capability of the detergent was improved after addition of lipase, which indicates the possible use of lipases for ecofriendly detergent formulation.

The profiled properties of lipase produced by the local isolate *B. cereus* HSS could be offered as a potential tool for commercialization and applications, where the cold-adaptive lipase introduce a low energy requirement for its optimum activity comparing to the traditional means that need high energy for optimum temperature and activity, which in turn would be the matter of interest. A complementary work concerning cloning of the purified lipases for further applications shall be conducted in the soon future.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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