

Production and Characterisation Partial Lipase of *Bacillus halodurans* CM1 Mutant for Biodetergen

Arina Aisyah^a, Trismilah^{b*}, Wibowo Mangunwardoyo^a and Dadang Suhendar^b

^aDepartment of Biology, Faculty of Mathematics and Natural Sciences,
Universitas of Indonesia, Depok-16424, Indonesia

^bAgro Industrial and Biomedical Technology Development Laboratory,
Agency for the Assessment and Application of Technology, Serpong-15314, Indonesia

(received October 25, 2018; revised March 23, 2019; accepted March 26, 2019)

Abstract. This study aims to produce lipase of the *Bacillus halodurans* CM1 mutant and its assess partial characteristics, performed in Bora and Bora modified medium. The purification was conducted using Ultrafiltration (UF), ammonium sulfate (AS) and polyethylene glycol (PEG). Results revealed that the highest purity lipase of *B. halodurans* CM1 mutant was 1.49-fold from the UF-AS-dyalisis, with a molecular weight of 35.7-37.4 KDa. The optimum condition of lipase enzyme was achieved at pH 7 and temperature 50 °C, relatively stable at pH 7-8 and temperature 30-70 °C. Mg²⁺, Ca²⁺, Zn²⁺, Mn²⁺, Fe²⁺ and K⁺ ions of concentrations, 1 mM to 10 mM increased enzyme lipase activity. The Km value was 0.23 mg/mL and Vmax 4.07 U/mL. Lipase was stable with the addition of a detergent concentration of 1-2% (69.60-57.10%), and with the washing test, the enzyme capable of hydrolyzing oil on cloth is 8.40%.

Keywords: *B. halodurans*, CM1 mutant, characterisation, lipase

Introduction

Lipase is an enzyme capable of catalysing hydrolysis and long chain synthesis of acylglycerols (tri-acylglycerol acyl-hydrolases) (EC 3.1.1.3), Andualema and Gessesse (2012). It is widely used in scientific testing i.e. cosmetic production, medical diagnosis, chemical analysis and bio-detergent industry, Cherif *et al.* (2011).

Bacteria are used to manufacture enzymes they produces a high degree of activity, neutral or alkaline optimum pH, as well as thermostable. Thermophile bacteria are the source of thermostable enzymes, and proteins derived from thermophilic micro-organisms are useful in biotechnology applications due to the high stability. Stability of lipases at high temperatures have a high reactivity, where an increase in substrate and product solubility, as well as a decrease in the viscosity present the risk of contamination. The bacteria of the genus *Bacillus* are capable of producing lipase (Balan *et al.*, 2012; Deive *et al.*, 2012).

Preliminary research conducted by the BPP Technology team, the bacteria isolated from hot springs, Cimanggu, west Java, had a molecular identification with 16SrDNA as a *Bacillus halodurans* CM1, with a ability to generate extra cellular enzymes, Ulfah *et al.* (2011). The pro-

*Author for correspondence; E-mail: trismilah@bppt.go.id

duction of lipase by *B. halodurans* CM1 was carried out at pH 9.00 and temperature 50 °C (Unpublished data).

The study with *B. halodurans* CM1 mutated with gamma radiation at doses of 0.1-0.4 KGy was performed according to the Internal Report (2016), however the lipase activity was obtained lower than the mutation resulted using N-methyl-N'- nitro-N-nitrosoguanidine (NTG) with a concentration of 0.1 mg/mL and incubation of 1-3 h. The NTG mutation resulted in *B. halodurans* CM1 gave the highest lipase activity was 5.13 U/mL.

Lipases applied have a wide ranged of pH and temperature stability, good selectivity and specificity to various substrates, and be easy to isolated. Enzyme characterisation needs to be carried out to understand the potential and be applied on an industrial scale Djafar *et al.* (2010).

The aim of this study is to produce lipase of *B. halodurans* CM1 mutant and to test their pH, temperature, metal ion and detergent. The calculation of molecular weight was performed and the kinetic enzyme calculated by using the Lineweaver-Burk equation. The application test for the lipase mutant was performed by assessing the stability of lipase, in addition to the detergent and enzyme ability test in hydrolysing the oil on cloth and the washing test.

Materials and Methods

Materials commonly used in the bioprocess of lipase production include; lipases that have been concentrated with stirred-cell ultrafiltration (Uicon) [Amicon], UF-ammonium sulfate (USA) [Merck], UF-polyethylene glycol (PEG) [Merck]; SDS-PAGE [Bio-Rad], and metal ions (1 and 10 mM MnSO₄ [Merck]; 1 and 10 mM ZnSO₄ [Merck 1 and 10 mM]; CaCl₂ [Merck]; 1 and 10 mM KCl [Merck]; 1 and 10 mM MgSO₄ [Merck] and FeSO₄ [Merck].

Lipase production. To build a seed culture, one loop of bacteria was in-oculated on liquid LB medium pH 9.00 with a working volume of 50 mL seed culture in Erlenmeyer 250 mL, agitation 200 rpm, a temperature 50 °C and incubated for 18-24 h.

Ten percent of the seed cultivation was used as the total starter LB medium pH 9.00 with a working volume of 100 mL in 500 mL Erlenmeyer, which were then incubated at 50 °C, agitation 200 rpm, until optical density (OD) reached 0.6-0.8 (2-4 h), before being inoculated into 10% of the total out put medium volume.

Shake culture of production medium Bora and Bora (2012) were modified with 0.5% PO and 0.09% CaCl₂ incubated in 3 L Erlenmeyer with a working volume of 500 mL (total working volume 1 L), agitation 200 rpm at 50 °C for 18 h. After 18 h, the cultures were harvested by mixing the two, and centrifuged at 3800 rpm for 30 min at 4 °C. The supernatant was taken as a crude enzyme (Ghaima *et al.*, 2014; Mokodongan, 2013).

Assay for lipase activity. The lipase activity test was conducted based on a modification from Li *et al.* (2014). The substrate was made of 25% olive oil, 1.50% polyvinyl alcohol (PVA), and homogenised with reverse osmosis (RO). A substrate of 5 mL was taken and added to 0.05 M tris-HCl buffer solution of pH 8.00 of 4 mL, then 1 mL of enzyme. Incubation was performed at 37 °C, 150 rpm for 20 min, before the sample was added with 5 mL of methanol, titrated with 0.05 M NaOH. The amount of enzyme needed to release one μmol of free fatty acid per minute under the experimental conditions were defined as one unit of lipase activity.

Partial purification. Stirred-cell ultrafiltration (UF). The 30 KDa filter membrane was installed in the UF device, and 30 psi of pressure was applied. The membranes were pre-treated, followed by 200 mL of crude enzymes being inserted into the tank at 100 rpm. Sampling was performed on crude enzymes prior to the

UF process and at 10× concentration, according to modification of Syed *et al.* (2010).

Ammonium sulfate precipitation. The enzyme with a stratified fractionation (20-80%), was slowly added to ammonium sulfate when mixed with the magnetic stirrer. Lipase 10 mL was prepared in a beaker and ammonium sulfate was added to the enzyme based on the ammonium sulphate Table. Ammonium sulphate of 1.07 g was slowly added into the enzymes, where it was mixed until dissolved, then the enzymes were incubated for 1 h. The enzymes were centrifuge at 3800 rpm for 30 min. The precipitated was added with 5 mL of 0.05 M tris-HCl buffer pH 7.00 for dialysis and the supernatant of the centrifugation product was calculated in volume to be used for the subsequent fraction. Membranes for dialysis were first pre-treated, Pratama (2015), one side of the membrane was tied, and the precipitated of ammonium sulfate dissolved with the buffer solution was poured into the dialysis membrane, the other side was also tied. The membrane was then immersed in 0.05 M tris-HCl buffer pH 7.00 with 1 L, rotating at 70 rpm at 18-20 °C overnight (18-24 h). Samples after dialysis, tested for activity and protein levels, Borkar *et al.* (2009).

Polyethylene glycol (PEG). Pre-treatment was performed on 12 KDa cellophane membrane, fastening on one side and filling in 10 mL enzymes sample on the other side for subsequent binding. A container already dusted with PEG 20 KDa was prepared, and the sample containing membranes were put in a container and then sprinkled with PEG until all the membranes had been coated and conditioned at 4 °C. The enzyme was concentrated until the retentate volume reached half the initial volume.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide gel was made using the mold, and after the gel was formed, it was removed and installed in the electrophoresis device tank. The 1× running buffer was poured into the tank until the specified limit. Each 12 μL sample was added with 5×3 μL sample buffer. The samples (15 μL) and markers (5 μL) were boiled in water for 1 min. Samples and markers were further fed into electrophoretic gel wells and the electrophoresis device was given a voltage of 150-200 V. After the process was complete, the gel was released and rinsed with miliQ, before being soaked and heated in a microwave for 1 min, repeated three times. The gel was then immersed in page blue and

heated in a microwave for 30 seconds, before it was incubated on top of thermo shaker for 1 h, at 300 rpm, room temperature (25-30 °C). After 1 h, the gel was rinsed and immersed in RO, and incubated overnight (18-24 h). The molecular weight was estimated by measuring the distance of the sample migration marked by the band and the distance of the sample tracer migration when electrophoresed (the bottom of the gel) as illustrated by Muchtaromah *et al.* (2012).

Profile of pH and lipase temperature. Determination of pH profile lipase of *B. halodurans* CM1 mutant was performed using an enzyme activity test at different pH levels (pH 6, 7, 8, 9, 10, 11 and 12). It was performed by titration method Li *et al.* (2014) with modification, and the buffer was used, ie 0.05 M phosphate buffer pH 6 and 7, 0.05 M Tris-HCl buffer pH 8 and 9, and 0.05 M glycine-NaOH buffer pH 10, 11 and 12. Incubation of the activity test at temperature 50 °C, 150 rpm, was conducted for 20 min. The profile temperature of the enzymes were determined at the optimum pH obtained with the determination of pH profiles consisting of varying temperatures (30, 40, 50, 60, 70 and 80 °C).

Stability of pH and lipase temperature. The effect of pH on stability lipase of *B. halodurans* CM1 mutant was measured by incubating the enzyme in pH buffer 6, 7, 8, 9, 10, 11 and 12 at room temperature for 90 min, then sampling each activity. The effect of temperature on the stability of the enzyme was measured by incubating for 1.5 h (sampling every 30 min) at 30, 40, 50, 60 and 70 °C and then tested its activity, as modified from Li *et al.* (2014).

Influence of metal ion. The influence of metal ions on lipase activity of the *B. halodurans* CM1 mutant was determined by testing enzyme activity containing 1 mM and 10 mM Mn²⁺, Zn²⁺, Ca²⁺, K⁺, Mg²⁺ and also Fe²⁺ (Mokodongan, 2013; Ghorri *et al.* 2011).

Km and Vmax. The rate of lipase in binding to the substrate was determined by analyzing the Km and

Vmax values, obtained by testing the enzyme activity at different concentrations of titration substrates. Variations of the substrate concentration used were 1, 2, 8, 12, 16 and 20%. The data of the enzyme activity test results were then plotted by the Lineweaver-Burk equation, Ratnayani *et al.* (2015) modified.

Stability in detergents. The stability of the enzyme to the addition of detergent was measured by the modified method of Cherif *et al.* (2011), where incubation of the lipase of *B. halodurans* CM1 mutant was mixed with added detergent concentrations of 1, 2, 3, 4 and 5% at 37 °C, 180 rpm for 60 min, then its enzyme activity at pH and optimum temperature were teste.

Washing test. WA washing test was performed based on Li *et al.* (2014) method: cloth was cut in 4×4 cm squares and soaked in boiling chloroform for 5 min. The cloth was then dried overnight at room temperature, and then weighed (Wa). The cloth was dropped on both sides by oil dissolved in acetone (100 µL/mL), and weighed again. It was then dried for 15 min at room temperature, immersed in an enzyme dissolved with 0.05 M phosphate buffer pH 7 (1:1), and incubated at 30 °C, 180 rpm for 1 h. The cloth was dried overnight at room temperature then weighed (Wc):

$$\text{Oil lost (\%)} = \frac{W_b - W_c}{W_b - W_a} \times 100$$

Results and Discussion

Lipase purification. The basic lipase activity of *B. halodurans* CM1 mutant increased from 9.50 U/mg to 10.38 U/mg following the concentration of stirred-cell ultrafiltration (UF). The results were comparable to the report of Balan *et al.* (2012) study, in that the ultrafiltration method was able to increase the specific activity of lipase *Geobacillus thermodentrificans* by 3% (Table 1).

Due to method of salting in and salting out precipitation of ammonium sulphate take place. The solubility of

Table 1. Partial purification lipase of *Bacillus halodurans* CM1 mutant

Steps of partial purification	Total protein (mg)	Total activity (Unit)	Spesific activity (Unit/mg)	Yield (%)	Purity (fold)
Crude enzyme	50.00	475.00	9.50	100	1.00
Ultrafiltration	5.78	60.00	10.38	12.63	1.09
UF – AS – Dialysis	0.65	9.19	14.12	1.94	1.49
U F - PEG	0.30	3.50	11.82	0.74	1.25

UF = stirred-cell ultrafiltration; AS = ammonium sulfate/(NH₄)₂SO₄ precipitation; PEG = polyethylene glycol.

protein will increase as the salt concentration increases (salting in), while a continuous addition of salt will result in decreased protein solubility (salting out), meaning that the protein is almost completely precipitated. Lipase of *B. halodurans* CM1 mutant from ultrafiltration (UF) was concentrated with ammonium sulphate until it reached a fraction of 20%, then continued dialysis increased specific activity from 10,38 U/mg to 14,12 U/mg. The increased activity of the enzyme, in accordance to the research of lipase from sea bacterial isolates of Pelabuhan Panjang, Bandar Lampung by Nurhasanah and Herasasi (2008), reported that concentration by ammonium sulphate increased enzyme activity from 0.21 U/mL to 3.50 U/mL.

Lipase from *B. halodurans* of CM1 mutant concentration with UF was also achieved by using polyethylene glycol (PEG). The dialysis membrane used had a pore size of 12000, while PEG measures 20000. PEG will cause molecules in enzymes smaller than 12000 to be attracted to the membrane, however it could not enter because the size is greater than 20000. Lipase of *B. halodurans* mutant CM had increased specific activity from 10,38 U/mg to 11,82 U/mg. Another study conducted by Padilha *et al.* (2012) reported that lipase activity from *Burkholderia cepacia* increased when concentrated using PEG.

SDS-page. Figure 1 suggests that protein bands appear in all samples except the crude enzyme. UF, UF-AS-dialysis and UF-PEG enzyme samples have one band each between the 30 KDa and 45 KDa markers.

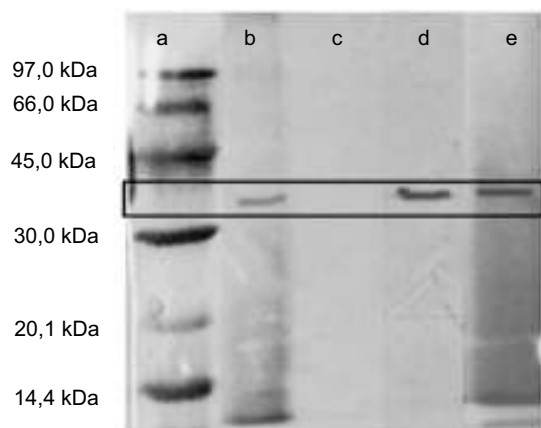


Fig. 1. SDS-Page results: a = Marker; b = UF sample; c = Crude enzyme sample; d = UF-AS-Dia-lysis sample; e = UF-PEG.

Estimation of molecular weight range lipase of 30-45 KDa, the genus *Bacillus* also has a molecular weight ranging between 19-40 KDa (Rabbani *et al.*, 2015; Shah and Bhatt, 2012; Sangeetha *et al.*, 2010).

Profile of pH and temperature. The optimum conditions for lipase of *B. halodurans* mutant CM1 were determined on the basis of the highest activity achieved, that being at pH 7 and temperature 50 °C, (Fig. 2-3).

The decreasing activity occurred at the basic pH after optimum pH was obtained, at pH 11 and 12, where no enzyme activity was detected, and at a high pH, where irreversible denaturation will occur. At low levels, hydrolysis occurs in unstable peptide bonds, and changes

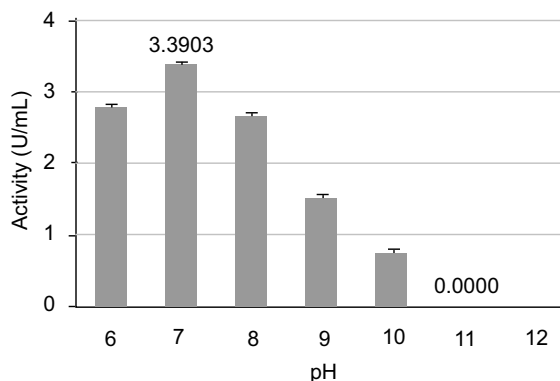


Fig. 2. The pH profile of lipase of *B. halodurans* CM1 mutant, pH 7 = phosphate buffer; pH 8 & 9 = Tris-HCl buffer; pH 10, 11 and 12 = buffer glycine-NaOH; activity of enzyme at T = 37 °C, incubation 20 min.

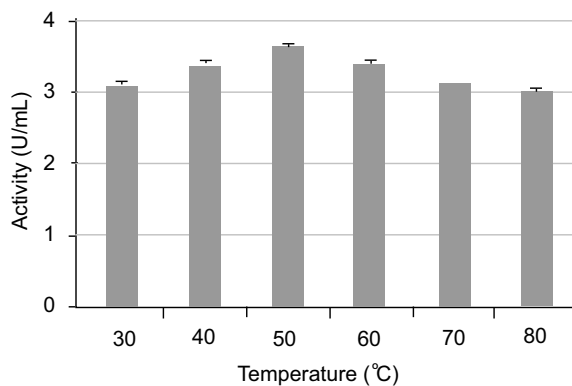


Fig. 3. The temperature profile of lipase *B. halodurans* CM1 mutant, enzyme activity at = 37 °C, pH = 7, incubation 20 min.

in the enzyme activity that was affected by pH occurred due to the enzyme ionization changes.

When enzyme production, temperature controls the synthesis of enzymes at the time of transcription of mRNA and protein translation, high-temperature protein stability is established, Bora and Bora (2012). The increase in temperature to the optimum is due to the increased kinetic energy that accelerates the movement and rotation of the enzyme molecules and substrate, thus increasing the collision frequency which provides a second chance to react. Above the optimum temperature the enzyme activity decreases, possibly due to a conformational change in the protein structure so that the reactive group may experience resistance to enter the active site of the enzyme, Tunggal *et al.* (2014).

Stability of pH and lipase temperature. The lipase activity of *B. halodurans* CM1 mutant is stable at pH 7-8 (Fig. 4). In general, bacterial lipase has an optimum pH of neutral or alkaline and is stable at pH 4-11, Qamsari *et al.* (2011). Wahyuni (2016) has confirmed that lipase derived from yeast is stable at pH 7-9, the lipase of *B. coagulans* BTS-3 was stable at pH 8-10.5.

Figure 5 showed that the activity lipase of *B. halodurans* CM1 mutant is relatively stable at 30 °C and drops at 40-50 °C before becoming stable again at 60-65 °C. The lipase being stable at high temperature (> 50 °C) is most likely due to polyamines in protein structures. In addition, the amount of hydrogen bond, increased salt bridges (sulphides), hydrophobic interactions, and high thermophilic amino acid proportions also affect

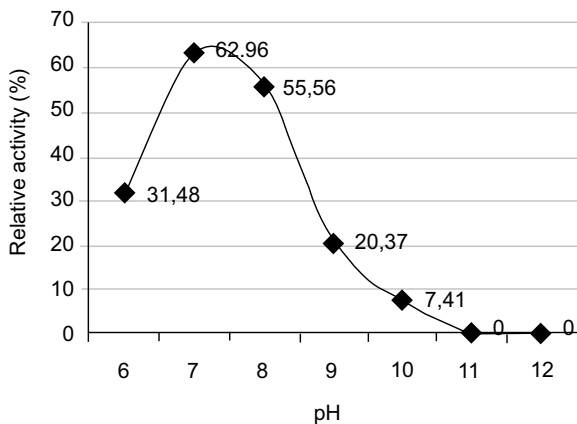


Fig. 4. Effect of pH on lipase activity stability, incubation time 90 min, analysis of enzyme activity at pH 7 and temperature 50 °C.

lipase stability against temperature, Bora and Bora (2012).

Influence of metal ion. All the metal ions tested were able to increase the lipase activity of *B. halodurans* CM1 mutant as shown in Fig. 6. Ion Ca^{2+} provided the highest activity at a concentration of 10 mM (5.20 U/mL). In addition to Ca^{2+} , Zn^{2+} ions also provide a high activity, either at concentrations of 1 mM or 10 mM (5.13 U/mL).

The enzymes require certain metal ions to increase activity by functioning as a cofactor for enzymes that can stabilize the binding to the substrate. The Ca^{2+} ions can induce changes in the conformation of enzyme

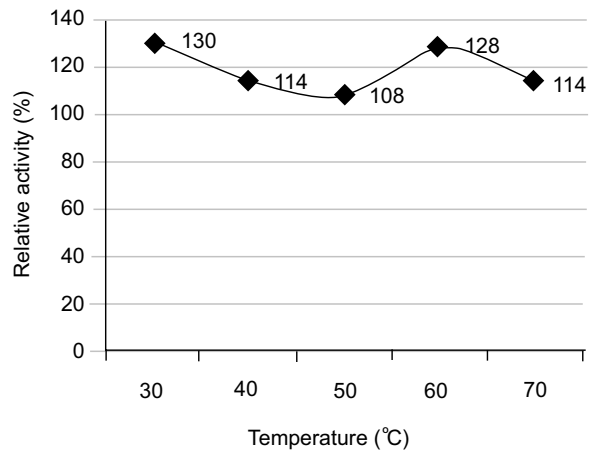


Fig. 5. Effect of temperature on lipase activity stability, incubation time 90 min, enzyme activity analysis at pH 7 and temperature 50 °C.

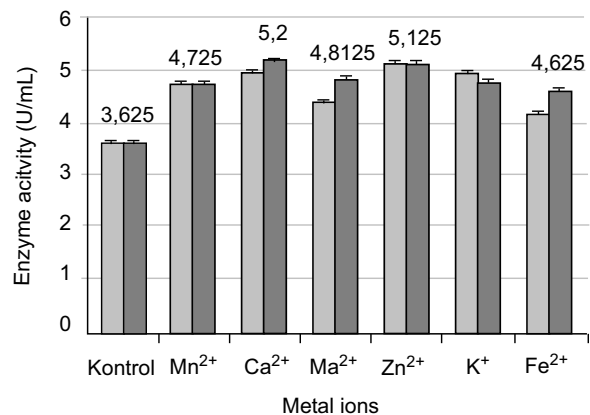


Fig. 6. Effect of addition of 1 mM and 10 mM metal ions to lipase activity.

structures to become more stable, thus increasing enzyme activity, Iqbal and Rahman (2015).

Km and Vmax. The data lipase of *B. halodurans* CM1 mutant activity test on various substrate activities were plotted on a graph, with the equation $y = 0.0561 + 0.2458x$ and a K_m value of 0.23 mg/mL and V_{max} of 4.07 U/mL. The smaller the K_m value, the higher the affinity for the substrate, thus indicating that a lower substrate concentration was required to reach the maximum catalytic reaction rate (V_{max}), Dali *et al.* (2011). Nurhasanah (2008) obtained K_m of 0.07 mg of substrate/mL and V_{max} of 1.51 U/mL of lipase with bacteria isolated from seawater.

Stability of detergent and washing test. The stability test lipase of *B. halodurans* CM1 mutant is illustrated in Fig. 7. The relative activity of enzyme was decreased along with an increase in detergent concentration. Lipase mutant showed stable additions of detergent concentration 1-2% (69.64-57.14%). The activity lipase decreased by almost 93%. An addition of detergent could cause a loss of lipase activity because it alters the structure of tertiary, however, it may also maintain lipase activity and stability by inhibiting lipase agglomeration, Li *et al.* (2014).

The result of the washing test of lipase enzyme on the cloth was found to lose 8.40% of oil content, where the control was only able to remove 2.40%. The washing test aims to determine the ability of lipase in hydrolyzing oil on the cloth, therefore the addition of *B. halodurans* CM1 mutant affects and the oil hydrolysis capability of the cloth even though it was still very low. The

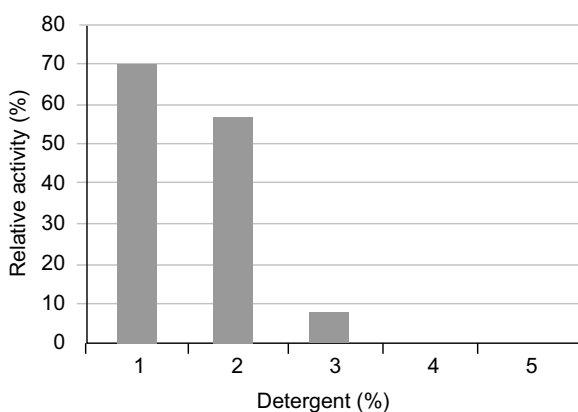


Fig. 7. Relative activity lipase of *B. halodurans* CM1 mutant against detergent with 45 min incubation time.

amount of oil lost from the cloth in the presence of enzyme addition was still higher than without the addition of the enzyme (control).

Conclusion

It can be concluded that lipase mutant *B. halodurans* CM1 activity can be increased by purification, where the SDS page can estimate the molecular weight. The optimum condition of lipase enzyme was achieved at pH 7 and temperature 50 °C, whereas stability was observed at pH 7-8 and temperature 30-70 °C. The highest Ca^{2+} ions increased lipase activity and was stable with the addition of detergent concentration of 1-2%. The washing test concluded that 8.40% is the enzyme capable of hydrolysing oil on cloth.

Acknowledgment

This research was supported by the Center for Bio-industrial Technology (PTB), Agency for the Assessment and Application of Technology (BPPT), which provided funding and laboratory facilities to conduct this study.

Conflict of Interest. The authors declare no conflict of interest.

References

- Andualema, B., Gessesse, A. 2012. Microbial lipases and their industrial applications: Review. *Biotechnology*, **11**: 100-118.
- Balan, A., Ibrahim, D., Rahim, R.A., Rashid, F.A.A. 2012. Purification and characterisation of a thermostable lipase from *Geobacillus thermodenitrificans* IBRL-nra. *Enzyme Research*, 1-7, doi:10.1155/2012/987523.
- Bora, L., Bora, M. 2012. Optimization of extracellular thermophilic highly alkaline lipase from thermophilic *Bacillus* sp. isolated from Hot Spring Arunachal Pradesh, India. *Brazilian Journal of Microbiology*, **43**: 30-42.
- Borkar, P.S., Bodade, R.G., Rao, S.R., Khobragde, C.N. 2009. Purification and characterisation of extracellular lipase from a new strain-*Pseudomonas aeruginosa* SRT 9. *Brazilian Journal of Microbiology*, **40**: 358-366.
- Cherif, S., Mnif, S., Hadrich, F., Abdelkafi, S., Sayadi, S. 2011. A newly high alkaline lipase: an ideal choice for application in detergent formulations. *Lipid in Health and Disease*, **10**: 221-228.
- Dali, S., Patong, A.R., Jalaluddi, M.N., Parenrengi, P.A.

2011. Pemurnian dan karakterisasi enzim lipase dari *Aspergillus oryzae* pada kopra berjamur. *Journal of Natur Indonesia*, **14**: 26-31.
- Deive, F.J., Mari'a, C., Alvarez, Paloma Mor'n, M.A., ngeles Sanroma'n, Mari'a, A. Longo. 2012. A process for extracellular thermostable lipase production by a novel *Bacillus thermoamylovorans* strain. DOI 10.1007/s00449-011-0678-9. *Bioprocess Biosyst Engineer*, **35**: 931-994.
- Djafar, F., Purwadaria, T., Sinurat, A.P. 2010. Isolation of endophytic bacteria from palm oil fruits and characterization of their lipases. *Microbiol Indonesia* **4**: 69-74.
- Ghaima, K.K., Mohamed, A.I., Mohamed, M.M. 2014. Effect of some factors on lipase production by *Bacillus cereus* isolated from diesel fuel polluted soil. *International Journal of Science and Research Publication*, **4**: 1-5.
- Ghori, M.I., Iqbal, M.J., Hameed, A. 2011. Characterization of novel lipase from *Bacillus* sp. isolated from tannery wastes. *Brazilian Journal of Microbiology*, **42**: 22-29.
- Iqbal, S.A., Rehman, A. 2015. Characterisation of lipase from *Bacillus subtilis* I-4 and its potential use in oil contaminated wastewater. *Brazilian Arch of Biology and Technology*, **58**: 789-797.
- Li, Xiao-Lu., Zhang, Wen-Hui., Wang, Ying-Dong., Dai, Yu-Jie., Zhang, Hui-Tu., Wang, Yue., Wang, Hai-Kuan, Lu, Fu-Ping. 2014. A high-detergent performance, cold-adapted lipase from *Pseudomonas stutzeri* PS59 suitable for detergent formulation. *Journal of Molecular Catalysis B: Enzymatic*, **102**: 16-24.
- Mokodongan, R.S. 2013. Karakterisasi dan purifikasi lipase ekstraseluler dari *Bacillus subtilis* DB-104 serta penggunaannya sebagai biokatalis reaksi transesterifikasi. *Thesis*, Universitas, Indonesia.
- Muchtaromah, B., Sumitro, S.B., Soemarno, S.T. 2012. Isolasi dan karakterisasi protein 100 KDa dari membran kelapa spermatozoa kambing. *Journal of Experimental of Life Science*, **2**: 13-19. Indonesia.
- Nurhasanah, H.D. 2008. *Pemurnian Enzim Lipase Dari Bakteri Lokal Dan Aplikasinya Dalam Reaksi Esterifikasi*. Prosiding. Universitas Lampung, Bandar Lampung, pp. 267-277, Indonesia.
- Padilha, G.S., Santana, J.C.C., Alegre, R.M., Tambourgi, E.B. 2012. Extraction of lipase from *Burkholderia cepacia* by PEG/phosphate ATPS and its biochemical characterisation. *Brazilian Arch of Biology and Technology*, **55**: 7-19.
- Pratama, L. 2015. Isolasi, karakterisasi dan produksi lipase dari kapang indogenous. *Thesis*, Sekolah Pascasarjana Institut Pertanian Bogor, Bogor, Indonesia.
- Qamsari, E.M., Kermanshahi, R.K., Nejad, Z.M. 2011. Isolation and identification of a novel, lipase-producing bacterium, *Pseudomonas aeruginosa* KM110. *Iranian Journal of Microbiology*, **3**: 92-98.
- Rabbani, M., Shafiee, F., Shayegh, Z., Sadeghi, H.M.M., Shariat, Z.S., Etemadifar, Z., Moazen, F. 2015. Isolation and characterisation of a new thermo-alkalophilic lipase from soil bacteria. *Iranian Journal of Pharmaceutical Research*, **14**: 901-906.
- Ratnayani, K., Laksmiwati, A.A.I.A.M., Sudiarto, M. 2015. Penentuan laju reaksi maksimal (Vmax) dan konstanta Michaelis-Menten (Km) enzim lipase pankreas pada substrat minyak kelapa, minyak sawit, dan minyak zaitun. *Journal of Kimia*, **9**: 93-97, Indonesia.
- Sangeetha, R., Geetha, A., Arulpandi, I. 2010. Concomitant production of protease and lipase by *Bacillus licheniformis* VSG1: production, purification and characterization. *Brazilian Journal of Microbiology*, **41**: 179-185.
- Shah, K.R., Bhatt, S.A. 2011. Purification and characterization of lipase from *Bacillus subtilis* Pa2. *Journal of Biochemistry and Technology*, **3**: 292-295.
- Syed, M.N., Iqbal, S., Bano, S., Khan, A.B., Qader, S.A., Azhar, A. 2010. Purification and characterisation of 60 kD lipase linked with chaperonin from *Pseudomonas aeruginosa* BN-1. *African Journal of Biotechnology*, **9**: 7724-7732.
- Tunggala, S., Natsir, H., Soekanto, N.H. 2014. *Optimasi Produksi Enzim Lipase Dari Bakteri Sumber Air Panas Tamalantik*, Mamasa, Sulawesi Barat. Prosiding. Universitas Hasanuddin, Makassar, Indonesia.
- Ulfah, M., Helianti, I., Wahyuntari, B., Nurhayati, N. 2011. Characterisation of new thermo alkalophilic xylanase-producing bacterial strain isolated from Cimanggu Hots Spring, West Java, Indonesia. *Microbiology Indonesia*, **5**: 139-143.
- Wahyuni, G. 2016. Pemurnian dan karakterisasi lipase dari yeast M2 sebagai biodeterjen. *Thesis*. Sekolah Pascasarjana Institut Pertanian Bogor, Bogor, Indonesia.