Cloning gene encoding lysophospholipase from *Bacillus halodurans* CM1 to *Escherichia coli* DH5α

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**Abstract.** Enzyme is a biocatalyst that is widely used in industry, for example detergent, pharmaceutical, food or oil purification. One of the most widely using enzymes for oil purification is lysophospholipase. As much as 50% of the needs of industrial enzyme are obtained from microorganisms. However, enzyme productivity of these wild type microbial strains is usually limited and cannot be applied in industry, so a genetic engineering is necessary. Cloning gene encoding for lysophospholipase was once performed in *Aspergillus niger* and *Cryptococcus neoformans*, but has never been conducted from alkaloithermophilic bacteria, such as *Bacillus halodurans*. *Bacillus halodurans* CM1 is an isolate from Badan Pengkajian dan Penerapan Teknologi (BPPT). Previous research has shown that these bacteria have lipase enzymes, but the study about their properties have not been conducted. This study aims to clone the gene lysophospholipase from *Bacillus halodurans* CM1 to *Escherichia coli* DH5α using the pGEM-T easy vector. The recombinant plasmid is sequenced. The gene fragment encoding lysophospholipase was successfully obtained with size 783 base pairs and 100% similarity with gene encoding lysophospholipase from *Bacillus halodurans* C-125 (No access GenBank: BA000004.3).

**Keywords:** *Bacillus halodurans* CM1, cloning, lysophospholipase

1. Introduction

Enzymes are widely used as biocatalyst in industry, such as detergents, medicines, food, and oil refining. Industrial enzymes can be obtained from plants, animals, and microorganisms. As much as 50% or more of these enzymes need to be obtained from microorganisms, because microbial enzyme is more stable. Enzymes that widely used in the oil refining industry are lipase and phospholipase [1]. Phospholipase, especially lysophospholipase can be more easily hydrolyzed than lipase [2]. Purification of the oil caused by a transesterification reaction, which causes the oil free contaminants. If transesterification reaction is performed in alkaline conditions, it can avoid the foaming. In addition, the use of enzymes in oil purification industry causes the oil free from harmful chemicals. However, the use of enzymes is limited because of the availability and price. Therefore, genetic engineering is required to produce high amounts of enzymes [2].

The lysophospholipase enzyme has been applied in oil refining process [2]. Lysophospholipase from *Aspergillus niger* has also been cloned and expressed in *Pichia pastoris* [3]. Coe et al. [4] has also been cloned lysophospholipase gene from *Cryptococcus neoformans*. However, cloning of gene encoding lysophospholipase from alkaloithermophilic bacteria, such as *Bacillus halodurans* has never been conducted. *Bacillus halodurans* CM1 is an isolate from Badan Pengkajian dan Penerapan Teknologi (BPPT) from hot spring sediment in Cimanggu, West Java. The bacteria have a similarity of 99% with 16S rRNA of *Bacillus halodurans* C-125 [5]. Previous research has shown that these
bacteria have lipase enzymes, but the study about their properties have not been conducted. The aim of this study is to clone the gene of lysophospholipase from *Bacillus halodurans* CM1 to *Escherichia coli* DH5α using the pGEM-T easy vector.

2. **Experimental method**

2.1. **Medium**
Culture of *Bacillus halodurans* CM1 in Horikoshi medium. Culture of recombinant *Escherichia coli* DH5α by previous study used LB medium contain ampicillin, X-GAL, and IPTG.

2.2. **Extraction genome of Bacillus halodurans CM1**
Extraction of the genome of *Bacillus halodurans* CM1 was performed using phenol-chloroform extraction method with modifications [6]. The visualization of extraction genome was captured in agarose 1 % by electrophoresis.

2.3. **Amplification fragment gene encoding lysophospholipase**
Amplification of gene fragments encoding lysophospholipase using primer was specifically designed based on sequences of genes encoding lysophospholipase from *Bacillus halodurans* C-125 on the site [http://www.genome.jp](http://www.genome.jp). The amplification was performed using KAPA Extra Hot Start Taq DNA polymerase based on the protocol of KAPA [7]. The result of extraction genome was visualized in agarose 1 % by electrophoresis.

2.4. **Transformation plasmid pGEM-T easy to Escherichia coli DH5α**
Ligation of the PCR fragment into pGEM-T easy vector using T4 DNA Ligase was carried out by protocol of [8]. Plasmid pGEM-T easy that contains lysophospholipase gene was transformed into a competent cell *Escherichia coli* DH5α by heat shock methods [9]. Screening of transformant was performed by screening blue-white that used LB agar containing ampicillin with a concentration of 100 mg/mL, isopropyl β-D-1-thiogalactopyranoside (IPTG) 0.1 M, and 5-bromo-4-chloro-3 -indolyl-beta-D-galactopyranoside (X-GAL) 4 % as medium. The culture was incubated overnight at 37 °C. The colour of positive colonies that contains plasmid with gene encoding lysophospholipase are white, while the colour of negative colonies are blue. Positive colonies was cultured in a liquid LB medium containing ampicillin for extraction plasmid DNA.

2.5. **Extraction plasmid DNA of Escherichia coli DH5α recombinant**
Extraction plasmid DNA isolation from positive colonies of recombinant *Escherichia coli* DH5α was performed by the alkali method [10]. Purification of the extracted plasmid DNA was performed using qualitative precipitation method PEG [11]. The extracted plasmid was also confirmed by digestion using the enzyme Eco RI [Fermentas]. The plasmid that has been confirmed by digestion was delivered to First Base for sequenced using primer forward PUC M13 (−40) and primer reverse M13 (−20). Sequences was analyzed by Basic Local Alignment Search Tool (BlastN) and database in Genebank at website [http://www.ncbi.nlm.nih.gov/blast.cgi](http://www.ncbi.nlm.nih.gov/blast.cgi).

3. **Results and discussion**
The genomic DNA extraction results were visualized by a 1 % agarose gel and 10,000 bp shown in figure 1. Figure 1 shows band at > 10,000 bp. According to Takami *et al.* [12], the size of the whole genome of *Bacillus halodurans* C-125, which has a 99 % similarity with *Bacillus halodurans* CM1, is 4,202,353 base pairs. Therefore, the genome can be extracted by the Saito and Miura method [6] and does not contain contaminants [13]. The result of amplification gene by specific primer can be seen in figure 2. Figure 2 shows there are thick band between 750 bp and 1.000 bp in lanes 1, 2, and 3.

The result of ligation can be seen from white colonies, which can grow on the transformation plate. There are 102 white colonies as well as satellites around the colony on the transformation plate. It causes the formation of the satellite in long incubation period and low concentration of ampicillin. Clone 1 and 3 are used for further analysis for plasmid extraction. Visualization of plasmid extraction can be seen in figure 3. The result of plasmid extraction show there are three bands on different sizes. The first band at ± 10,000 bp, the middle band in the middle at the mark of ± 8.000 bp; and the third band
Figure 1. The visualization of genomic DNA extraction of *Bacillus halodurans* CM1

Figure 2. The visualization of gene amplification lysophospholipase

Figure 3. The visualization extraction of plasmid clones 1 and 3

Figure 4. The visualization of digestion with *EcoRI*

at the mark of ± 3.000 bp. The third band indicates three conformation of extracted plasmid, which is nicked, linear and super coiled [14]. Therefore, the recombinant plasmid cannot be sized before digestion performed with restriction enzyme. The result of using *EcoRI* restriction enzyme can be seen in figure 4. Figure 4 shows two bands at the mark ± 3.009 bp and ± 789 bp. The clones 1 and 3 that have been confirmed by digestion will be used for sequencing.

The sequenced of electropherogram shows the unambiguous peak. Pairwise alignment of the sequence can be seen in figure 5. Analysis on the aligned sequence with BLAST showed gene has 100% similarity with gene encoding lysophospholipase from *Bacillus halodurans* C-125. It can be concluded, gene had been cloned into plasmid pGEM-T easy.
Figure 5. Result of pairwise alignment with gene encoding lysophospholipase *Bacillus halodurans* C-125

4. Conclusions
Gene encoding lysophospholipase from *Bacillus halodurans* CM1 with size 783 bp were successfully cloned to *Escherichia coli* DH5α using pGEM-T easy vector.

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