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# Amplification of Thermostable Lipase Genes Fragment From Thermogenic Phase of Domestic Waste Composting Process

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**Abstract.** Lipases are lipolytic enzymes, catalyze the hydrolysis of fatty acid ester bonds of triglycerides to produce free fatty acids and glycerol. The enzyme is widely used in various fields of biotechnological industry. Hence, lipases with unique properties (e.g.thermostable lipase) are still being explored by variation methods. One of the strategy is by using metagenomic approach to amplify the gene directly from environmental sample. This research was focused on amplification of lipase gene fragment directly from the thermogenic phase of domestic waste composting in aerated trenches. We used domestic waste compost from waste treatment at SABUGA, ITB for the sample. Total chromosomal DNA were directly extracted from several stages at thermogenic phase of compost. The DNA was then directly used as a template for amplification of thermostable lipase gene fragments using a set of internal primers namely Flip-1a and Rlip-1a that has been affixed with a GC clamp in reverse primer. The results showed that the primers amplified the gene from four stages of thermogenic phase with the size of lipase gene fragment of approximately 570 base pairs (bp). These results were further used for Denaturing Gradient Gel Electrophoresis (DGGE) analysis to determine diversity of thermostable lipase gene fragments.

Key words : PCR, lipase, metagenomic approach, compost

## 1. Introduction

Lipase (triacylglycerol acylhydrolases , EC 3.1.1.3) is a lipolytic enzyme that functions to hydrolyze carboxylic ester bonds triglycerides to produce fatty acids and glycerol. Lipase is widely used in various fields such as food industry, detergents biology, medical fields, sewage treatment, organic synthesis, hydrolysis of fats and oils, modified fat, flavor enhancer in food processing, the resolution of racemic mixtures and chemical analysis, the leather industry, also in the pulp industry and paper [1, 2]. In addition, the lipase can also be utilized in increasing degradation of wastes containing fats and polyurethane. The broad applications of lipase is due to several things including: lipase has a broad substrate specificity, stable in organic solvents and possess high regio-selectivity [2].

Indonesia is a country that has a high biodiversity. The presence of geothermal area in natural and artificial widespread allow for obtaining thermophilic microbes with high diversity. Research to obtain thermophilic bacteria producing thermostable enzymes have been carried out through cultivation techniques. However, only less than 1 % of microbe have been successfully cultivated because of the limitations and the difficulty of finding a selective medium for microbial growth. One method that is currently done is metagenomic approach which involve isolation of microbial genomes directly from natural samples without cultivation. Metagenome approach is a relatively new method in an attempt to find the source of thermostable enzymes. Some studies of lipase obtained through metagenome approaches such as lipase from sediment [3], soil [4], and activated sludge [5]. Through this approach, it is possible to get different variants of the lipase enzyme which has been studied previously.

Compost is the artificial geothermal environment that consists of organic material from domestic waste. During the composting process, the organic components are biochemically decomposed by mesophilic and thermophilic bacteria under aerobic conditions, where the thermostable enzyme such as lipase plays an important role in this stage [6]. During the process, the compost will experience changes in temperature, pH, and the structure of organic materials [7]. Research on microorganisms on various types of compost has also been carried out and shows the diversity of microorganisms and enzymes produced in each phase of composting [8, 9].

Here we report, the presence of a lipase gene fragments from thermogenic phase of composting domestic waste treatment has been successfully amplified using primers internal lipase gene that were designed and reported in previous studies [10].

## **2. Materials and Methods**

### **3. Composting and sampling**

Compost sample was taken from composting process at TPS Sabuga, ITB, Bandung with the distance at around 1 km from the Biochemistry Laboratory. The composting process use domestic waste as compost material with traditional process. 1-2 kg compost of thermogenic phase at temperature around 45-67 °C was collected from about 30 cm of compost surface. Samples were immediately used for further analysis.

### **4. Preparation of sample**

Water extracts of the compost samples were prepared by shaking approximately 30 grams of the fresh sample in 270 ml of sterile distilled water with horizontal shaker for 30 minutes and then filtered. Water extract was re-filtered through a 0.22- $\mu$ m-pore-size cellulose membrane filter (Sartorius, Germany). Microbes pellet in the membrane was resuspended in STE buffer (10 mM Tris-HCl pH 8.0, 0.1 M NaCl, 1 mM EDTA) and then stored at -20 °C until used for DNA isolation.

### **5. Isolation of total DNA**

Total DNA from samples were extracted using the Power soil DNA isolation kit (MO BIO Laboratories, Inc., California) according the manufacture instruction. The DNA was stored at -20 °C until use.

### **6. Amplification of Thermostable lipase gene Fragments**

The partial of lipase genes was amplified by using a set of primers namely Flip-1a (5'TGATCG GCCACAGTCAGGG 3') and Rlip-1a with 39 base GC clamp incorporated (5'CgCCCgCCgCgCCCCgCgCCCggCCCgCCgCCCCgCCC GTTGATCTCGTCGATATGGTT 3'). PCR was performed by using Sso fast evagreen supermix according to the instruction provided from the manufacturer (Biorad). Amplification was performed with PCR within 35 cycles denaturing at 98 °C for 30 second, annealing at 55 °C for a 30 second, extension at 72 °C for a minute and final extension at 72 °C for 10 minutes. PCR product was then examined with ethidium bromide-stained agarose gel.

## **7. Results and Discussion**

Lipase is a class of hydrolases that play an important role in the industrial world. Based on amino acid sequence homology, lipase bacteria are classified into eight families [11]. Family I is a "true lipase" producing bacteria group consisting of 6 subfamily. Two of the four regions conserved in sub family 1.1 and 1.2 has been used to design primers which are containing catalytic triad of the amino acid lipase i.e serine and histidine. This internal primer was designed to amplify fragments of the lipase gene at around 570 bp.

Composting process are carried out in the Waste Sites (TPS) Sabuga, ITB, Jalan Taman Sari No. 73 Lebak Siliwangi, Bandung. Composting was done traditionally without using starter and aeration system otomatic. The compost pile is made with a length x width x height = 100 cm x 100 cm x 100 cm. The highest temperature was reach at the 18<sup>th</sup> day (67 °C) and then fell gradually until the maturation process was completed during the composting process. The compost profile and sampling points on the thermogenic phase can be seen in Figure 1.

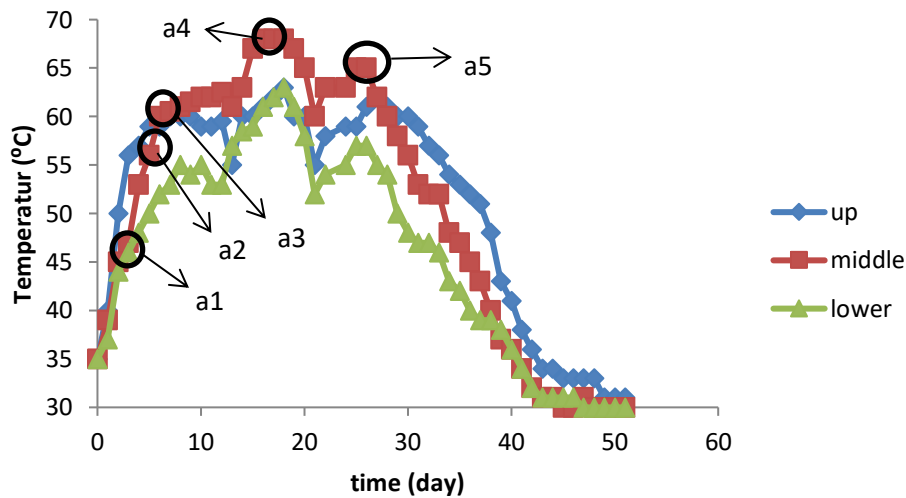


Figure 1. The compost profile and sampling point during thermogenic phase.  
(a1-a5 = sampling point at a1=45 °C, a2= 56 °C, a3= 60,5 °C, a4= 67 °C and a5= 65 °C)

In this research, the composts were taken from each sampling point, then suspended and filtered through Millipore 0,22 µm membrane to collect the cell (Figure 2A). The filtration result of compost samples was showed as blackish brown cell, indicating the high presence of humic acid in the samples [12]. Furthermore, a collection of microbial cells were resuspended to STE buffer and collected in 2 mL microtube to be stored at -20°C or to be extracted (Figure 2B).

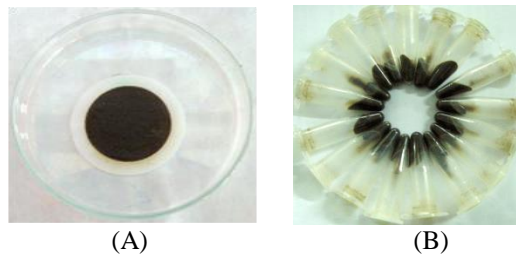


Figure 2. Cells compost which is a collection of microbial cells.  
(A) The cell using a membrane filtration  
(B) The cell was washed with STE buffer

Total community DNA from each samples was isolated from the cells and examined under agarose gel electrophoresis. It showed a single band on ethidium bromide-stained agarose gel (Figure 3A) and it has a relatively high purity in the absence of other bands and RNA were detected on the agarose gel. This DNA then used as template for amplification, in which the DNA template used is a mixture of compost microbe. We have successfully amplified the fragment by using primer pair Flip-1a and Rlip-1a that has been affixed with a GC clamp to amplify lipase gene fragment. Mixture of total community DNA has been successfully used as template to amplify the lipase gene. The PCR products were then tested with the agarose gel electrophoresis. Electrophoregram showed a single band at about 570 bp (Figure 3B).

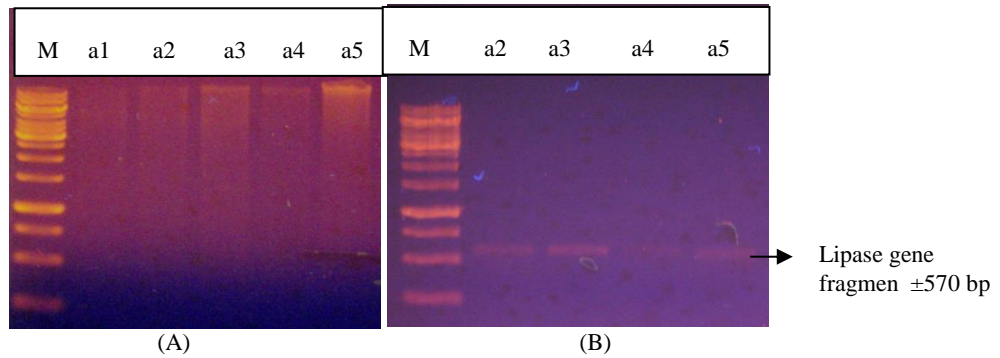


Figure 3. Electrophoregram of total DNA community and lipase gene fragment from some sampling point.  
 (A) Electrophoregram of total DNA community from some sampling point (M=marker 1kb DNA ladder; a1-a5 = total community chromosomal DNA from sampling point a1-a5)  
 (B) Electrophoregram of lipase gene fragment from some sampling point ((M=marker 1kb DNA ladder; a2-a5 = lipase gene fragment at sampling point a2-a5)

Based on the results of electrophoresis in 1% agarose with ethidium bromide addition (Figure 3B), the presence of DNA bands visible at 2,3 4 and 5 points (for 1 point not showed) of composting with the size of  $\pm 570$  bp. It shows that the primers designed has been successfully used to amplify lipase gene fragment of total comunity DNA from natural samples.

## 8. Conclusion

The designed primer was successfully used to amplify lipase gene fragment from total chromosomal DNA isolated from compost samples at some point in the thermogenic phase.

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