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Isolation of Local Lipolytic Isolate from Domestic Compost

Syifa F. Syihab[1], Fida Madayanti[1] and Akhmaloka[1*]

[1] Biochemistry Research Group, Faculty of mathematics and Natural Sciences, Institut Teknologi Bandung, Indonesia

*Corresponding Author: loka@chem.itb.ac.id

Abstract. Screening of lipolytic bacteria from domestic compost resulting an isolate namely AL17. Morphological analysis shows that the isolates were rod shape and belong to negative gram bacteria. The 16S rRNAs genes of the bacteria have been sequenced, and phylogenetic analysis showed that the isolates were close to genus *Pseudoxanthomonas*. The enzyme production was synchronized with bacterial growth and reached a maximum level during the late-stationary phase. The optimum pH and temperature of enzyme activity were at pH 9.0 and 60°C.The isolate also showed alcohol tolerance in medium containing 3% and 5% methanol. The ability of bacterial cells to tolerate methanol is an important cell characteristic that determines their use as a biocatalyst in transesterification and other industrial process.

Keywords: Thermostable lipase, Compost, Solvent tolerant

Introduction

Lipase is one of the most applicable biocatalyst in industries, this enzyme can catalyse both the hydrolysis and the synthesis of long-chain acylglycerols with high regioselectivity and enantioselectivity [1]. Microbial lipases have been applied in oleochemistry, detergents, paper, and food industry. Isolated thermostable lipases are playing important roles in industrial processes, because they are applicable in the enzymatic processing of lipids, even at high temperatures and alkaline condition [2]. Bacteria which are able to produce lipase can be found in various places, including dairy industries and oil wastes, hot springs and soils contaminated with oils [3, 4]. Thermostable enzyme can be isolated from thermal environment such as compost [5]. Decomposition of organic materials in the composting process was carried out by succession of microbial communities [6]. The composting process were consist of three major phases: mesophilic, thermophilic and maturation stages. Study by Madayanti *et. al.,* (2008) have successfully cultivated and collected 10 isolates from thermogenic phases (50-70°C) during composting process which showed lipolytic activity [7]. In this work we reported the isolation, identification and characterization of lipase producing bacteria from domestic compost.

Materials and Methods

Microorganism isolation from compost

Compost sample was taken from ITB Composting Unit, Bandung. Microorganism was isolated from the compost sample at the peak of thermogenic phase (72°C). Sample was diluted in 50 ml sterile normal saline. After shaking for 15 minutes, 1% of the suspension was transferred into medium of 0.5% yeast extract, 0.1% NaCl, 0.1% $CaCl_2$ and 0.5% lab lemco. The culture was incubated overnight at 55°C and 150 rpm aeration. Serial dilution was conducted towards the culture and followed with incubation in media with 2% bacto agar. The cultures was incubated overnight at 55°C. Isolation of bacteria were carried out by 4-quadrant streak plate method using the same media. Single colonies of various bacteria were obtained by replica plating method repeated at least three times. Morphological analysis of cell was undertaken by observing cell's shape morphology and Gram's staining [8].

Screening of Lipase-Producing Bacteria

Screening of lipase-producing bacteria was carried out using rhodamine B-olive oil agar plate method [9]. The bacteria was incubated at 55 °C for 2 days in agar plate contain 1% olive oil, 0.5% tween 80, 0.3% aquabidest and 0.2% rhodamine B. The lipase-

producing bacteria were identified by the orange fluorescent halos around the colonies when the plates were irradiated with UV illuminator.

DNA extraction

Chromosomal DNA from microorganisms were isolated using Klijn et. al., (1991) method with some modification [10]. DNA pellets were resuspense using 30μL nuclease free water and stored at 4°C. The obtained DNA solution is ready to use for PCR amplification.

Amplification and Sequencing of 16S rRNA Gene

The 16S rRNA genes of the five isolates were amplified by Polymerase Chain Reaction (PCR) technique [11]. The 16S rRNA gene of individual bacteria was amplified using a pair of universal primers, namely UniB1 (Univ1492R: 5'-GGTTAC(G/C)-TTGTTACGACTT-3') and BacF1 (Bac27F: 5'-AGAGTTTGA-TC(A/C)TGGCTCAG-3') (22-23). PCRs were performed by using Taq DNA polymerase according to the instructions provided by the manufacturer (Fermentas). The PCR products were verified by electrophoresis that conducted on 1% agarose in buffer TAE 1x using submerged horizontal electrophoresis cell (BioRad) for 45 minute at 70 volt. In order to obtain complete sequences of 16S rRNA genes, an automatic DNA sequencer (Macrogen, Korea) was employed based on direct sequencing method from PCR products using four pairs of PCR primers.

Sequence analysis

The electrophoregram data from the sequencing was firstly analysed by Seqman program. Homolog of each sequence were analysed using online software "NCBI-Blast", via the NCBI website [12].

Optimum pH and Temperature for Lipase activity

Lipase activity was measured using spectrophotometric assay with p-nitro phenyl laureate (PNPL) dissolved in acetonitrile at a concentration of 10 mM as substrate [13]. Subsequently, ethanol and potassium phosphate buffer (50 mM; pH 8.0) were added to final composition of 1:4:95 (v/v/v) of acetonitrile/ethanol/buffer, respectively. The cell-free supernatant (0.3 ml) was added to the substrate solution (0.9 ml) and then, the mixture was incubated at 55°C. After 15 min, enzyme activity was measured by monitoring the change in absorbance at 405 nm that represents the amount of released p-nitro phenol (PNP). One unit of lipase activity is defined as the amount of enzyme releasing 1 μmol PNP per min under the assay conditions. The optimum pH on lipase activity was examined at 55°C using 50mM buffer with a pH range from 5.0 to 11.0. Buffer system include phosphate buffer (pH 4.0 to 8) and glycine-NaOH buffer (pH 9 to 11). Effect of temperature on lipase activity was determined by measuring lipase activity at 40 to 80 °C.

Alcohol-Tolerant Bacteria

Culture of cell were grown in medium containing 3 and 5% of methanol at 55°C with shaking at 150 rpm. The number of cells were count as optical density (OD) per hour at 600nm.

Results and Discussions

Isolation of Lipolytic Bacteria

Sampling was carried out randomly from Composting Unit at Institut Teknologi Bandung, West Java. Using cultivation method, we discover 50 viable isolates. The isolates was then incubated using agar plate with addition of olive oil as carbon source. After 2 days of incubation, the lipase-producing bacteria were showing orange fluorescent halos around the colonies when the plates were irradiated with UV illuminator. Screening of lipolytic bacteria resulting an isolate of bacteria with lipase activity, this isolates was assigned as AL17. The morphological observation to the five isolates was using gram staining method [8] and the observation was using optical microscope with 1600x magnification showed that the isolate have rod-like shape and also gram negative microorganism

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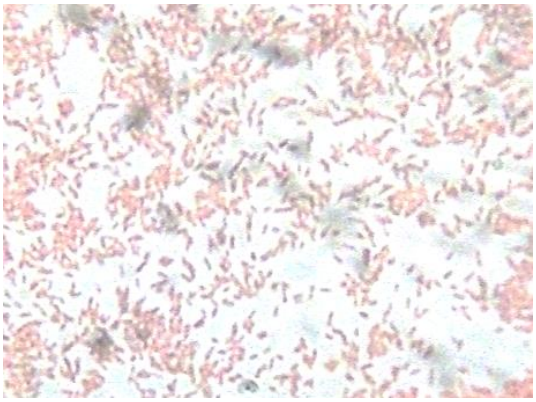

Figure 1. Cell morphology of AL17

The Sequence and Phylogenetic of the Isolate

Amplification of 16S rRNA genes towards the isolates were performed by PCR technique, while the nucleotide sequences were determined using sequencing method. PCR amplification of 16S rRNA gene have successfully amplified full-sized gene with the length about 1500 bp (Fig. 2). The sequence alignment of each 16S rRNA gene was carried out by BlastN to build the list of known bacteria in the GenBank with high similarity (99–100% identities) to the isolate. Alignment of the gene showed that the isolate have high similarity to *Pseudoxanthomonas taiwanensis*.

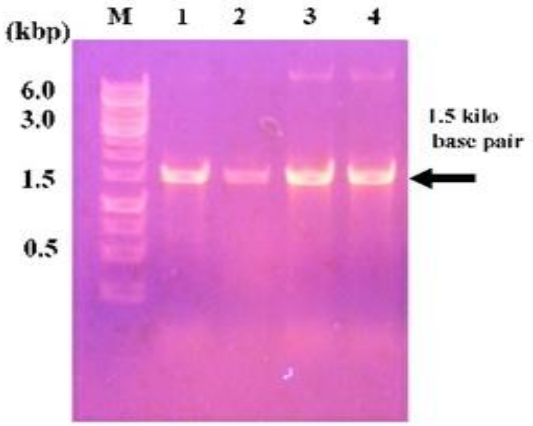

Figure 2. PCR amplifications result of 16S rRNA gene of the isolate. The assigned lane number is as follow: M=DNA marker, 1 – 4 = AL17

Optimum pH and Temperature for Lipase Activity

In effort to discover the optimum time of lipase production, we have observed the optical density and lipolytic activity during the incubation. The isolate started the logarithmic phase after 6 hours of incubation, and after 10 hours it begin to enter the stationary phase. The optimum lipolytic activity of AL17 was occurred after 17 hours of incubation and significantly dropped at 18 hour (Fig. 3).

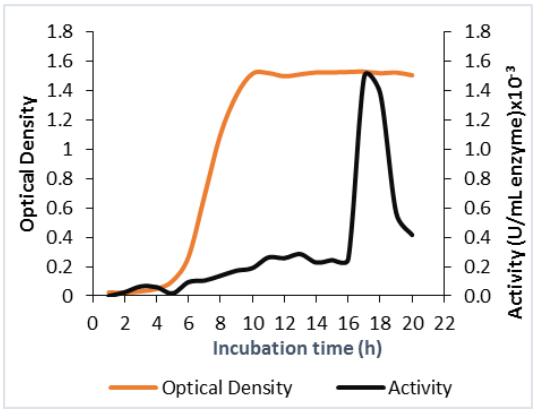

Figure 3. Optimum time of lipase production

The lipase activity were tested using p-nitro phenyl laurate as the substrate. Optimum pH on lipase activity was studied from pH 4 to 11. Lipase showed high activity only in pH 9 (Fig. 4.), while most of lipases typically have optimum pH in the range of 7-14. Assay towards the optimum temperature for lipase activity was conducted from 40°C to 80°C. The result showed that extracellular lipase from AL17 has optimum activity at 60°C (Fig.5). Generally, lipases isolated from microorganisms show optimal activity at temperature ranges of 30-60°C. Based on the result, we suggested that AL17 secrete thermostable alkaline lipase.

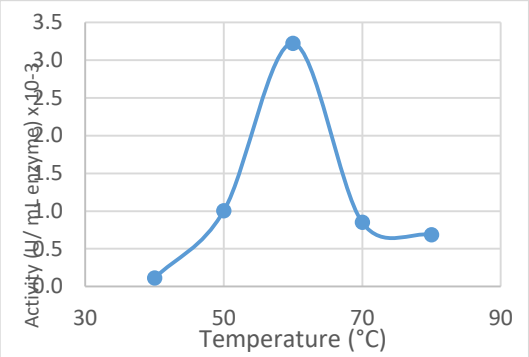

Figure 5.Optimum pH. Lipase from AL17 have optimum activity on pH 9.

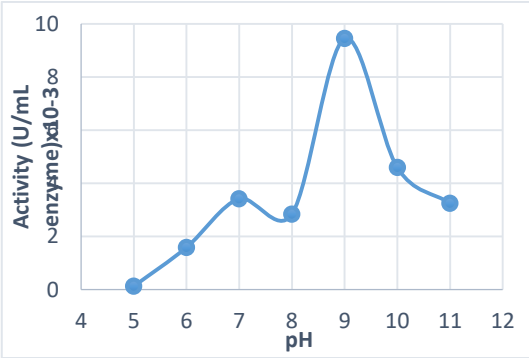

Figure 4.Optimum Temperature. Lipase from AL17 have optimum activity at 60°C.

Methanol Tolerance of the Isolates

The methanol tolerance ability of the cell were investigated using 3% and 5% (v/v) methanol. Methanol was added to the media from the first hour of incubation. We also incubate the bacteria using media without methanol as control. The number of cells were count based optical density (OD) per hour at 600nm. After 10 hour of incubation, we determine the growth rate of the isolate based on optical density at logarithmic phase. The result shows that the growth rate were decreased on 3% and 5% methanol. The addition of 3% methanol decrease 44% of growth rate, while 5% methanol decrease 56% of the cell growth rate after 10 hour incubation. Solvent tolerant bacteria have been reported as negative gram bacteria like *Pseudomonas aeruginosa, P.* Putida, and *Escherichia coli* [14]. It is common thought that organic solvents is toxic to living microorganism because of their accumulation in hydrophobic cell membranes [15]. Proposed cell mechanism to overcome the presence of solvent is by adaptive alterations of the membrane fatty acids and phospholipid head group composition so that reduced membrane permeabilization [16].

Conclusion

Isolation and identification of AL17 reveal that the cell have rod-like shape, Gram's negative and potential to produce lipase. Based on 16S rRNA analysis the isolates were belonged to genus *Pseudoxanthomonas.* The addition of 3% and 5% methanol decreased about 50% of the growth rate.

Acknowledgement

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