



CLONING OF ALKALINE THERMOSTABLE LIPASE GENE FROM LOCAL THERMOPHILIC MICROORGANISM

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INTRODUCTION

Lipase is enzyme that catalyze hydrolysis and synthesis of long chain acylglycerols. The use of lipases for a variety of biotechnological applications is rapidly and steadily increasing. Among the desirable characteristics that commercially important lipase should exhibit, alkali tolerance and thermostability are the main factors¹. To meet this end, there is a continuous search for sources of highly active lipolytic enzymes with specific stability to pH, temperature, ionic strength and organic solvents. Indonesia is one of the most tectonically active area in the world, has highly potential as habitat of thermophiles. Numbers of thermophiles from hot springs around west java have successfully been cultivated in our laboratory². Some of these bacteria are identified as a lipase producer strains³.

MATERIAL AND METHODS

ISOLATION OF CHROMOSOMAL DNA.

Chromosomal DNA from local thermophilic microorganism has been isolated using Klijn methods with some modification. The pellet cells were suspended in 10 mM Tris HCl buffer (pH 8.0) containing 8 mg/ml of lysozyme and incubated at 37°C for 1 h, the cells were lysed by adding lysis buffer containing 2% SDS, 0.8 mg/ml proteinase K and 200 mM EDTA pH 8.0. The lysis process was carried out by incubation at 50°C for 30 min. Ice cold potassium acetate and acetic acid glacial mixed solution were added and the denatured proteins were precipitated by centrifugation. Supernatants were mixed with an equal volume of chloroform isoamylalcohol

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(24:1, v/v). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h. The pellet of crude nucleic acids were obtained by centrifugation at 16.000 g for 20 min at room temperature, washed twice with cold 70% ethanol, and resuspended in sterile deionized water.

AMPLIFICATION AND SEQUENCING OF 16S RRNA GENE.

The amplification of 16S rRNA genes was carried out as described previously by Baker et al. (2001). The 16S rRNA genes were amplified using two set of primers, 5'-AGAGTTTGATC(A/C)TGGCTCAG-3' and 5'-GGTTAC(G/C)TTGTTACCTGCCGGA-3'. All of PCR products were subjected to DNA sequencing. Sequencing was carried out in an ABI PrismR 3100 Genetic Analyzer (Applied Biosystems) by the Macrogen Sequencing Service (Korea).

AMPLIFICATION OF LIPASE GENE

Chromosomal DNA was used as template for amplification fragment gene of lipase. Several primers are designed to amplify DNA fragment which encode lipase gene. Internal primer amplified fragment of lipase gene inside coding region. We used 2 conserved regions of lipase as basic information to design internal primer. The primers were designed based on nucleotide sequences encoding amino acid sequences of lipase from same group of our lipase producer strain, which is *Geobacillus*⁴. Nucleotide sequence of this fragment gene is used as basic information for external primer design by comparing it with lipase sequences available in GeneBank. Primer external is designed to gain the whole gene of lipase.

RESULT AND DISCUSSION

Some of local thermophilic microorganisms from hot spring around West Java have been reported to produce alkaline thermostable lipases³. Identification of the isolates based on its 16S rRNA sequence showed that all isolates were belongs to *Geobacillus* genus but different species. We have cloned the gene encoding thermostable lipase based on PCR amplification process using 2 set of primers, internal and external primers. There are two conserved amino acid sequences in lipase gene. One region was at around one of three lipase's catalytic residues, amino acid serin. This region is known as conserved pentapeptide which always in form of Gly-X-Ser-X-Gly. Another region is homologous region to the oxyanion hole region of the *Pseudomonas glumae* lipase located at 60-108 aa upstream of the conserved pentapeptide⁵. This region was recognized by the presence of a short hydrophobic region (6 aa) upstream of a moderately conserved His-Gly

(HG) dipeptide. Internal primer amplified fragment between these 2 conserved regions with length of approximately 300 bp. Alignment analysis of nucleotide sequence from these fragments showed that lipase from isolates can be classified into 2 groups. The first group was closely related to lipase from *Geobacillus* sp. SF1 (96%). The second group was closely related to lipase from *Geobacillus thermoleovorans* (97%). Nucleotide sequence of the fragment genes were used for designing the external primer by comparing it with lipase sequences available in GeneBank. From the alignment analysis using Blastn program in <http://ncbi.nlm.nih.gov>, we only found 6 lipase sequence which has high homology with our lipase and contain upstream and downstream of its coding region. Region around 20 bases upstream the start codon and region 30 bases after the termination codon (TAA) were used as bases for external primer design. External primer has successfully amplified whole coding region of lipase genes with approximately length of 1300 bp. Alignment analysis of amino acid sequence showed that the lipase from local isolates closely related to lipase from *Geobacillus stearotherophilus* (98%). The cloning of these genes into expression vector are still in progress.

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