KLONING DAN OVEREKSPRESI GEN PENGKODE LIPASE DARI BAKTERI *Alcaligenes* sp. JG3

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INTISARI

Telah dilakukan karakterisasi urutan nukleotida, kloning dan overekspresi dari gen pengkode lipase *Alcaligenes* sp. JG3 serta analisis protein lipase JG3 secara komputasi. Penelitian ini bertujuan untuk mengkonfirmasi dan menganalisis urutan nukleotida gen pengkode lipase JG3, mendapatkan DNA rekombinan gen lipase JG3 dengan plasmid vektor dan mendapatkan enzim lipase JG3 murni dan uji aktivitasnya.

Karakterisasi nukleotida lipase JG3 diperoleh dari amplifikasi gen dengan PCR menggunakan primer yang didesain berdasarkan gen lipase dari *Alcaligenes faecalis* subsp. *faecalis* NCIB 8687 dan dari hasil analisis sekuensing terhadap DNA lipase rekombinan (pGEM-T/LipJG3). Kloning DNA lipase rekombinan tersebut dilakukan melalui proses kejutan panas dengan inang *E. coli* JM109 dan untuk proses overekspresi digunakan inang *E. coli* M15[pREP4] yang diinduksi dengan isopropil β-d-1-thiogalaktopiranosida dan dipurifikasi menggunakan matriks Ni-NTA. Selanjutnya, analisis terhadap urutan nukleotida, asam amino dan struktur 3-dimensi dari lipase JG3 dilakukan menggunakan pendekatan berbasis komputasi (*in silico*).

Hasil karakterisasi menunjukkan bahwa gen lipase JG3 terdiri atas 1071 nukleotida atau 356 asam amino. Urutan tersebut memiliki kemiripan terhadap protein ATP *Binding Cassette* (ABC) transporter dan lipase hingga 96 dan 93% secara berurutan. Kloning gen lipase JG3 berhasil dilakukan dengan efisiensi transformasi sebesar $1{,}54 \times 10^3$ CFU. Enzim lipase hasil overekspresi memiliki berat molekul mencapai 46 kDa dan mampu menghidrolisis triasilgliserol minyak zaitun dengan aktivitas spesifik sebesar 1,175 U/mg. Hasil analisis protein dari proses *in silico* menunjukkan bahwa lipase JG3 memiliki karakter protein ABC transporter dengan adanya urutan nukleotida yang bersifat lestari yakni LSGGQQRVAIA yang merupakan ciri dari keluarga ABC transporter. Dari penelitian ini, telah diprediksi satu struktur 3-dimensi protein yang diduga adalah protein lipase JG3.

Kata kunci: gen lipase JG3, PCR, DNA rekombinan, ABC transporter

CLONING AND OVEREXPRESSION OF LIPASE-ENCODING GENE FROM *Alcaligenes* sp. JG3 BACTERIUM

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ABSTRACT

Nucleotides sequence characterization, cloning, overexpression of lipase encoding gene from *Alcaligenes* sp. JG3 and analysis of lipase JG3 protein has been accomplished. This study aims to confirm and analyze the lipase JG3 encoding gene, to obtain the recombinant DNA of lipase JG3 gene within vector plasmid, to obtain the purified lipase JG3 enzyme and to determine its activity.

Lipase JG3's nucleotides characterization was done from the gene amplification using PCR with the primer pair which was designed based on lipase gene from *Alcaligenes faecalis* subs. *faecalis* NCIB 8687 and from sequencing analysis of lipase recombinant DNA (pGEM-T/LipJG3). Cloning of the recombinant DNA was performed using heat shock method and used *E. coli* JM109 as hosts while for the overexpression, *E. coli* M15[pREP4] was used as hosts which was induced by isopropyl β-d-1-thiogalactopyranoside then purified using Ni-NTA matrices. Furthermore, the analysis towards nucleotides and amino acids sequence and also 3-dimensional structure of lipase JG3 were carried out using *in silico* approach.

The nucleotides characterization showed that lipase JG3 gene consists of 1071 nucleotides or 356 amino acids. Those sequence have similarities towards ATP Binding Cassette (ABC) transporter protein and lipase protein up to 96 and 93% respectively. The cloning of lipase JG3 was successfully done with the calculated efficiency of transformation is 1.54×10^3 CFU. The overexpressed lipase enzyme has molecular weight of 46 kDa and is able to hydrolyze triacylglycerol from olive oil with the specific activity up to 1.175 U/mg. The results from *in silico* analysis of lipase JG3 protein showed that it has the characteristic of ABC transporter protein because it has the sequences of LSGGQQRVAIA which features as the conserved sequence from ABC transporter family. From this study, a 3-dimensional structure of putative lipase JG3 protein has been proposed.

Keywords: lipase JG3 gene, PCR, recombinant DNA, ABC transporter