

ABSTRAK

ISOLASI OPEN READING FRAME (ORF) LYTIC POLYSACCHARIDE MONOOXYGENASE DARI *Bacillus* sp. T3 DAN ANALISIS PRODUKNYA MELALUI IN SILICO

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Lytic Polysaccharide Monoxygenase merupakan enzim pengoksidasi ikatan glikosidik β -1,4 pada polisakarida yang berperan penting dalam depolimerisasi senyawa kitin dan selulosa. Enzim ini memiliki potensi pemanfaatan untuk menghasilkan biofuel dan senyawa kimia lain yang bersumber dari polisakarida kitin dan selulosa. Penelitian ini bertujuan untuk mengamplifikasi *open reading frame* (ORF) gen LPMO dari *Bacillus* sp. T3 dan melakukan analisis *in silico* produk dari ORF tersebut. Amplifikasi ORF gen LPMO berhasil dilakukan dengan menggunakan metode *Polymerase Chain Reaction* (PCR). Identifikasi ORF dilakukan dengan menggunakan BLASTx kemudian produk dari ORF tersebut dianalisis menggunakan MEGA7 dan ExPASy ProtParam. Blast NCBI digunakan untuk menganalisis struktur primer, sedangkan PSIPRED digunakan untuk menganalisis struktur sekunder, Swiss Model digunakan untuk prediksi struktur tersier dan PyMOL 2.2 digunakan untuk visualisasi struktur tersier protein LPMO. Berdasarkan hasil amplifikasi *putative* ORF dan analisis menggunakan Blastx diketahui ORF gen LPMO memiliki ukuran 621 bp. Berdasarkan hasil ExPASy ProtParam diketahui bahwa LPMO dari *Bacillus* sp. T3 memiliki panjang sekuen 206 asam amino dengan rasio asam amino muatan positif dan negatif sebesar 0,957, rasio Arg/Lys sebesar 0,591 serta rasio Gly/Ala sebesar 0,59. Analisis struktur primer dan sekunder protein membuktikan bahwa protein LPMO dari *Bacillus* sp. T3 memiliki karakteristik yang berbeda dengan LPMO dari bakteri lainnya. Perbedaan ini terletak pada residu katalitik H125 yang berubah menjadi D125. Adapun struktur tersier protein LPMO dari *Bacillus* sp. T3 memiliki kesamaan dengan struktur tersier protein LPMO dari *B.amyloliquefaciens* (PDB : 2YOX).

Kata kunci: amplifikasi, analisis *in silico*, *Bacillus* sp. T3, LPMO

ABSTRACT

ISOLATION OPEN READING FRAME (ORF) LYTIC POLYSACCHARIDE MONOOXYGENASE FROM *Bacillus* sp. T3 AND IN SILICO CHARACTERIZATION OF THE ORF PRODUCT

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Lytic polysaccharide monooxygenase (LPMO) is an enzyme that depolymerize cellulose or chitin through oxidative mechanism leading to subsequent cleavage of the β -1,4-glycosidic bond. This enzyme has important role for biofuel and other chemical compounds production from cellulose or chitin polymers. This work was aimed to amplify the open reading frame (ORF) of the LPMO encoding gene from *Bacillus* sp. T3 and characterize the properties of the product of the ORF by a means of *in silico* analysis. Amplification of the ORF LPMO encoding gene was successfully carried out using the Polymerase Chain Reaction (PCR) method. The ORF confirmation was done using BLASTx then the ORF products were analyzed using MEGA7 and ExPASy ProtParam. The NCBI blast was also used to analyze the primary structure, while the secondary and tertiary structures of LPMO were analyzed by using PSIPRED and Swiss Model, respectively. To visualize the tree dimensionally structure of LPMO, the PyMOL 2.2 suite was employed. BlastX analysis of the DNA sequencing further confirmed that the PCR product was the open reading frame of LPMO. The size of the ORF was 621 bp in length. Amino acids deduction from the nucleotides sequence of the ORF, which was 206 amino acid residues, was further analyzed by using ProtParam. Based on the amino acids composition, the LPMO of *Bacillus* sp. T3 had the positive and negative amino acid residue ratio of 0.957, Arg/Lys ratio of 0.591 and Gly/Ala ratio of 0,59. Analysis of primary and secondary structures showed that LPMO from *Bacillus* sp. T3 had distinct characteristics compared to the LPMO of other bacteria. This difference lies in the catalytic residue of H125, in which the H residue changes to D125. The tertiary structure of LPMO protein from *Bacillus* sp. T3 was similar to the tertiary structure of LPMO protein from *B.amyloliquefaciens* (PDB: 2YOX).

Keywords: amplification, in silico analysis, *Bacillus* sp. T3, LPMO