INTISARI

ISOLASI, KLONING, DAN ANALISIS *IN SILICO OPEN READING FRAME* (ORF) *LYTIC POLYSACCHARIDE MONOOXYYGENASE* (LPMO) DARI *Serratia marcescens* LS1

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Selulosa dan kitin merupakan polisakarida rekalsitran yang memiliki struktur amorf dan kristalin yang sulit untuk didegradasi Salah satu enzim yang dapat mendegradasi selulosa dan kitin adalah *Lytic Polysaccharide Monooxygenase* (LPMO) yang dapat mendegradasi polisakarida rekalsitran dengan cara memutus ikatan β-1,4 glikosidik. Tujuan dari penelitian ini yaitu untuk isolasi, analisis *in silico*, serta kloning *Open Reading Frame* (ORF) LPMO.ORF LPMO diisolasi menggunakan metode *Polymerase Chain Reaction* (PCR) lalu disekuensing dan dianalisis menggunakan BLASTx. Kemudian dilakukan analisis *in silico* yang meliputi analisis filogenetik, fisikokimia, dan struktur protein menggunakan ExPASy ProtParam, MEGA7, Swiss Model, dan PyMOL 2.2. ORF LPMO berhasil diisolasi dengan metode PCR yang berukuran ±558 bp. Analisis filogenetik menyatakan bahwa LPMO *Serratia marcescens* LS1 berkerabat dekat dengan *Serratia marcescens* CBP21 dan *Serratia marcescens* SMB2099. Analisis fisikokimia menunjukkan bahwa LPMO *Serratia marcescens* LS1 memiliki rasio basa/asam sebesar 1,43; rasio Arg/Lys sebesar 0,67; dan rasio Pro/Gly sebesar 1. LPMO *Serratia marcescens* LS1 memiliki satu domain katalitik dan terdiri dari 4 *α-helix* dan 8 *β-sheet*. Residu katalitik dari LPMO *Serratia marcescens* LS1 terdiri dari His15, His100, dan Phe173. Model struktur tersier dari LPMO *Serratia marcescens* LS1 memiliki orientasi yang sama dengan LPMO *Serratia marcescens* CBP21 (PDB: 2BEM) dengan nilai RMSD sebesar 0,062. Upaya kloning rekombinan DNA dari ORF LPMO pada penelitian ini belum berhasil, sehingga diperlukan kloning dan ekspresi dari ORF LPMO dari *Serratia marcescens* LS1 pada penelitian selanjutnya.

Kata kunci: *In silico*, kloning, *Lytic Polysaccharide Monooxygenase*, ORF, *Serratia marcescens* LS1.

ABSTRACT

ISOLATION, CLONING, AND IN SILICO ANALYSIS OF OPEN READING FRAME (ORF) LYTIC POLYSACCHARIDE MONOOXYGENASE (LPMO) FROM *Serratia marcescens* LS1

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Cellulose and chitin are recalcitrant polysaccharide that contain crystalline and amorphous structure and, difficult to be degraded. One of the enzyme that is able to degrade cellulose and chitin is Lytic Polysaccharide Monooxygenase (LPMO) by breaking the β-1,4 glycosidic bond. The aims of this study are to isolate the open reading frame (ORF) of LPMO from *Serratia marcescens* LS1, clone, and perform in silico analysis of LMPO. The ORF of LPMO was isolated and amplified by Polymerase Chain Reaction (PCR) method, then the PCR product was sequenced and analyzed using BLASTx. In this study, in silico analysis of phylogenetic analysis, physicochemical, build the model protein structure, and validation were done by using ExPASy ProtParam, MEGA7, Swiss Model, and PyMOL 2.2. The resulting amplification from LPMO of *Serratia marcescens* LS1 showed a band with the approximate size of 558 bp. Phylogenetic analysis showed that LPMO from *Serratia marcescens* LS1 was highly conserved with LPMO either from *Serratia marcescens* CBP21 and *Serratia marcescens* SMB2099. Physicochemical analysis showed that LPMO from *Serratia marcescens* LS1 had 1,43 acid/base ratio with the ratio of Arg/Lys and Pro/Gly were 0,67 and 1, respectively. Based on the model protein structure, LPMO from *Serratia marcescens* LS1 had one catalytic domain which consist of 4 α-helixes and 8 β-sheets. Catalytic residue of LPMO from *Serratia marcescens* LS1 consist of His15, His100, and Phe173. Tertriary structure of the LPMO has the same structure as LPMO from *Serratia marcescens* CBP21 (PDB: 2BEM) with an RMSD value of 0,062. Unfortunately, the DNA recombinant of LPMO in this study has not been successfully to be cloned, therefore, generating the DNA recombinant of LPMO from *Serratia marcescens* LS1 is necessary for further research to overcome the functional studies of LPMO.

Keyword: cloning, In silico, Lytic Polysaccharide Monooxygenase, ORF, *Serratia marcescens* LS1.