INTISARI

ISOLASI *OPEN READING FRAME XYLANASE* DAN KARAKTERISASI PRODUKNYA SECARA *IN SILICO* DARI *Bacillus* sp. T3

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Xylanase merupakan enzim glikosida hidrolase yang memiliki kemampuan dalam menghidrolisis ikatan 1,4-β-xylosidik. *Xylanase* berperan penting dalam proses degradasi polisakarida terutama hemiselulosa. Perlu dilakukan upaya untuk mengeksplorasi lebih banyak *xylanase* dari berbagai bakteri untuk mengidentifikasi karakter *xylanase* yang berpotensi untuk keperluan rekayasa. Pada penelitian ini bertujuan untuk mengisolasi *orf xylanase* dari *Bacillus* sp. T3 dan melakukan analisis karakter *xylanase* yang terekspresikan secara *in silico*. Analisis *in silico* meliputi analisis fisikokimia dan analisis struktural. *Open reading frame* (*orf*) *xylanase* dari *Bacillus* sp. T3 telah berhasil diisolasi dengan menggunakan metode *Polymerase Chain Reaction* (PCR) menghasilkan pita berukuran ± 1200 bp. Produk PCR kemudian digunakan untuk sekuensing DNA untuk analisis lebih lanjut. Analisis BLASTx menunjukkan bahwa orf menunjukkan nilai *identity* sebesar 77 hingga 85% pada *endo*-1,4-β-*xylanase* dari kelompok *Bacillus*. Produk orf selanjutnya dianalisis dengan menggunakan *ExPASy ProtParam*, MEGA7, dan *ExPASy SWISS MODEL*. *Xylanase* memiliki berat molekul 44,9 kDa dengan nilai pI 6,38. *Xylanse* dari *Bacillus* sp. T3 menunjukkan rasio Arg/Lys yang secara signifikan lebih tinggi daripada kelompok bakteri mesofilik, termofilik dan psikrofilik. Ini menunjukkan bahwa *xylanse* dari *Bacillus* sp. T3 lebih bersifat stabil daripada *xylanase* dari bakteri lainnya. Analisis struktural berdasarkan model 3D *xylanase* dari *Bacillus* sp. T3 menunjukkan bahwa *xylanase* memiliki situs pengikatan logam (Ca^{+}) yang terletak pada *carbohydrate binding module* (CBM). Residu katalitik yang dibentuk oleh Asp-24, Asp-163 dan Glu-226 memiliki orientasi yang mirip dengan *Arabinoxylan arabinofuranohydrolase Bacillus subtilis* BsAXH-m2,3.

Kata kunci : *orf,* isolasi, analisis *in silico*, *xylanase*, *Bacillus* sp. strain T3.

ABSTRACT

ISOLATION OF OPEN READING FRAME XYLANASE AND IN SILICO CHARACTERISATION OF THE ORF PRODUCT FROM *Bacillus* sp. T3

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Xylanase is a hydrolase glycoside enzyme which has an ability to hydrolyze 1,4-β-xylosidik bonds. Xylanase has played an important role for polysaccharide degradation especially hemicellulose. Efforts should be made to explore more xylanases from various bacteria in order to further identify their characters which have potentially useful for engineering purposes. Therefore, the objective of this work were to isolate open reading frame (orf) of xylanase from *Bacillus* sp. T3 and perform in silico analyses of the xylanase that might be expressed from the orf. In silico analysis that were carried out included physiochemical analysis and structural analysis. The orf of xylanase from *Bacillus* sp. T3 has been successfully isolated using Polymerase Chain Reaction (PCR) method which resulted band of ± 1200 bp. The PCR product was then used for DNA sequencing for further analyses. BLASTx analysis indicated that the orf exhibited 77 to 85% identity to the endo-1,4-β-xylanase of the Bacilus group. The orf product was further analsyses by using MEGA7, ExPASy ProtParam and ExPASy SWISS MODEL. The xylanase has molecular weight of 44.9 kDa with the pI value of 6.38. Xylanse from *Bacillus* sp T3 exhibited the Arg/Lys ratio which significantly higher than that of mesophilic, thermophilic and psychrophilic group. This indicating that the xylanse from *Bacillus* sp T3 was intriguingly more stable than that the xylanases from others bacteria. Structural analysis, based on the 3D model of xylanase from Bacillus sp. T3 showed that xylanase has a metal binding site (Ca^+) that located on a carbohydrate binding module. The catalytic residues which was formed by Asp-24, Asp-163 and Glu-226 had similar orientation to that of *Arabinoxylan arabinofuranohydrolase Bacillus subtilis* BsAXH-m2,3.

Keywords : orf, isolation, in silico analysis, xylanase, *Bacillus subtilis* strain T3.