

INTISARI

ISOLASI *OPEN READING FRAME* (ORF) 6-FOSFO- β -GLUKOSIDASE DARI *Bacillus* sp. T3 DAN KARAKTERISASI PRODUKNYA SECARA *IN SILICO*

SIH PIRENANINGTYAS

14/364084/PN/13558

Selulosa dapat dimanfaatkan di berbagai industri. Namun, proses degradasi selulosa menjadi monosakarida oleh endoglukanase dan eksoglukanase dihambat oleh keberadaan selubiosa. Oleh karena itu, 6-Fosfo- β -glukosidase memegang peranan penting karena dapat memotong ikatan 1,4- β -glikosidik dari selubiosa. Tujuan dari penelitian ini adalah untuk mengisolasi *Open reading frame* (ORF) 6-Fosfo- β -glukosidase dan melakukan karakterisasi secara *in silico* dari produk ORF tersebut. ORF 6-Fosfo- β -glukosidase dilakukan sekuensing dan dianalisis dengan menggunakan BLASTx. Kemudian, produk ORF tersebut dikarakterisasi dengan menggunakan ExpASy ProtParam, MEGA7, PSIPRED, Swiss Model, dan PyMOL 2.2. Hasil sekuensing menunjukkan bahwa *Bacillus* sp. T3 memiliki ukuran nukleotida sebesar 1392 bp. Analisis BLASTx menunjukkan bahwa 6-Fosfo- β -glukosidase memiliki *identity* sebesar 97% dengan 6-Fosfo- β -glukosidase dari grup *Bacillus*. Analisis filogenetik juga menyatakan bahwa 6-Fosfo- β -glukosidase *Bacillus* sp. T3 memiliki kekerabatan yang dekat dengan kelompok termofilik. Analisis fisikokimia dari produk ORF tersebut menunjukkan bahwa 6-Fosfo- β -glukosidase *Bacillus* sp. T3 memiliki rasio asam amino basa dan asam sebesar 0,774; rasio Arg/Lys sebesar 0,778; dan rasio Ala/Gly sebesar 0,68. Analisis struktural menunjukkan bahwa 6-Fosfo- β -glukosidase *Bacillus* sp. T3 masuk ke dalam famili GH1, yang memiliki bentuk topologi α/β TIM barrel. Dua residu katalitik dari 6-Fosfo- β -glukosidase *Bacillus* sp. T3, Glu-157 dan Glu-365, memiliki orientasi yang sama terhadap residu-residu yang sesuai dari 6-fosfo- β -galaktosidase *Geobacillus stearothermophilus* (4ZE5). Analisis struktural juga menunjukkan bahwa 6-Fosfo- β -glukosidase dari *Bacillus* sp. T3 memiliki dua residu penting dalam pengikatan fosfat, yaitu Lys-425 dan Tyr-427. Berdasarkan analisis tersebut, 6-Fosfo- β -glukosidase *Bacillus* sp. T3 memiliki karakteristik yang mengarah ke karakteristik kelompok bakteri termofil. Namun, perlu dilakukan uji eksperimental untuk mendukung analisis *in silico* dari 6-Fosfo- β -glukosidase *Bacillus* sp. T3.

Kata kunci : ORF, 6-Fosfo- β -glukosidase, *Bacillus* sp. T3, analisis *in silico*.

ABSTRACT

ISOLATION OF OPEN READING FRAME (ORF) 6-PHOSPHO-B-GLUCOSIDASE FROM *Bacillus* sp. T3 AND IN SILICO CHARACTERIZATION OF THE ORF PRODUCT

SIH PIRENANINGTYAS

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Cellulose can be used in various industries. However, the degradation of cellulose into monosaccharides by endoglucanase and exogucase is inhibited by the presence of cellulose. Therefore, 6-phospho- β -glucosidase plays an important role because it can cleaves 1,4- β -glycosidic bonds from cellobiose. The aims of this study were to isolate the open reading frame (ORF) of 6-phospho- β -glucosidase and perform in silico characterization the product of the ORF. The ORF 6-phospho- β -glucosidase was sequenced and subjected for BLASTx analysis. The ORF product was then characterized by using ExPASy ProtParam, MEGA7, PSIPRED, Swiss Model, and PyMOL 2.2. Sequencing results showed that the ORF of *Bacillus* sp. T3 had nucleotides size of 1392 bp. BLASTx analysis clearly showed that the ORF product was 6-phospho- β -glucosidase with 97% identity to the 6-phospho- β -glucosidase of *Bacillus* group. Phylogenetic analysis also indicated that 6-phospho- β -glucosidase of *Bacillus* sp. T3 was close to the thermophilic group. Physicochemical analysis of the ORF product showed that 6-phospho- β -glucosidase of *Bacillus* sp. T3 had base and acid amino acid ratio of 0,774; Arg/Lys ratio of 0,778; and Ala/Gly ratio of 0,68. Structural analysis indicated that 6-phospho- β -glucosidase of *Bacillus* sp. T3 belongs to the GH1 family, which had protein topology of α/β TIM barrel. The two catalytic residues of 6-phospho- β -glucosidase of *Bacillus* sp. T3, Glu-157 and Glu-365, were orient similar to the correspond residues of 6-phospho- β -galactosidase of *Geobacillus stearothermophilus* (4ZE5). Structural analysis also indicated that 6-phospho- β -glucosidase of *Bacillus* sp. T3 had two important residues for phosphate binding, namely Lys-425 and Tyr-427. Based on this analysis, 6-phospho- β -glucosidase *Bacillus* sp. T3 has characteristics that lead to the characteristics of thermophilic bacteria. However, an experiment is needed to support the in silico analysis of 6-phospho- β -glucosidase *Bacillus* sp. T3

Keywords : ORF, 6-phospho- β -Glucosidase, *Bacillus* sp. T3, in silico analysis.