



INTISARI

KLONING DAN ANALISIS *IN SILICO* ORF THERMOLYSIN-LIKE PROTEASE DARI *Bacillus* sp. T3

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Thermolysin merupakan enzim protease yang bersifat termostabil. Perlunya biaya yang besar dalam menemukan dan menumbuhkan bakteri termofilik menyebabkan munculnya upaya untuk mendapatkan protease termostabil dari bakteri mesofilik. Pada penelitian ini dilakukan kloning, dan analisis *in silico* ORF *thermolysin-like* protease dari *Bacillus* sp. T3. untuk mengetahui struktur dan karakter dari enzim tersebut. ORF gen penyandi *thermolysin-like* dari *Bacillus* sp. T3 telah berhasil dikloning dengan menggunakan metode *Polymerase Chain Reaction* (PCR). ORF *thermolysin-like* hasil kloning yang berukuran sekitar 1500 bp berhasil ditransformasikan ke dalam *E. coli* BL21 (DE3) dengan menggunakan plasmid pET28a. Hasil uji keberhasilan transformasi dilakukan dengan metode PCR koloni dan menghasilkan ukuran yang sama dengan hasil kloning sebelumnya. Analisis struktur dan karakter ORF *thermolysin-like* dari *Bacillus* sp. T3 menggunakan *BLAST*, *ExPASy ProtParam*, *MEGA6*, dan *PSIPRED*. Dari hasil analisis sekuen diketahui bahwa ORF *thermolysin-like* protease dari *Bacillus* sp. T3 memiliki panjang sekuen 506 asam amino, pI pada kisaran 7, mengandung jumlah asam amino muatan positif yang lebih banyak dibandingkan negatif dengan perbandingan rasio 1,019, dan rasio asam amino arginine dan lysine sebesar 0,368. Nilai-nilai tersebut mendekati nilai pada hasil analisis kelompok bakteri mesofil. Pada analisis secara struktural, ORF *thermolysin-like* protease *Bacillus* sp. T3 memiliki *zinc binding* site pada sisi aktif dengan residu katalitik berupa His-142, His-146, dan Glu-166. Dari keseluruhan analisis *in silico* yang telah dilakukan, dapat disimpulkan bahwa ORF *thermolysin-like* protease dari *Bacillus* sp. T3 memiliki karakter yang dekat dengan kelompok bakteri mesofil.

Kata kunci : ORF, thermolysin, *thermolysin-like* protease, *Bacillus* sp. T3, kloning, analisis *in silico*.



ABSTRACT

CLONING AND IN SILICO ANALYSIS OF ORF THERMOLYSIN-LIKE PROTEASE FROM *Bacillus* sp. T3

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Thermolysin is a protease enzyme which is thermostable. The need for a high cost in discovering and growing the thermophilic bacteria has led the efforts to produce thermostable protease of mesophilic ones. Therefore, this research was conducted in cloning and in silico analysis of ORF thermolysin-like proteases from *Bacillus* sp. T3 to find out the structure and character of these enzymes. ORF of thermolysin-like encoder gene from *Bacillus* sp. T3 was successfully cloned by using Polymerase Chain Reaction (PCR) method. ORF of thermolysin-like cloning results, measuring around 1500 bp was successfully transformed into *E. coli* BL21 (DE3) using plasmids pET28a. Analysis of transformed cells was done by using colony PCR method that had the same size as previous cloning result. Analysis of the structure and the character of the ORF thermolysin-like from *Bacillus* sp. T3 was done by using BLAST, ExPASy ProtParam, MEGA6, and PSIPRED. Sequence analysis results shows that ORF of thermolysin-like protease from *Bacillus* sp. T3 has a sequence of amino acids 506 long, pI in the range 7, contains more positive charges of amino acids than negative ones by ratio 1.019, and 0.368 for the ratio of arginine and lysine amino acids. These numbers are quite close to the value of the analysis results of mesophile bacteria groups. In structural analysis, ORF of thermolysin-like protease from *Bacillus* sp. T3 has a zinc binding site on the active side of the catalytic residues in His-142, HIS-146, and Glu-166. From overall in silico analysis that have been done, it can be inferred that the ORF thermolysin-like proteases from *Bacillus* sp. T3 has close characters with group of mesophile bacteria.

Keywords: ORF, thermolysin, thermolysin-like protease, *Bacillus* sp. T3, cloning, in silico analysis.