KLONING GEN PENYANDI PROTEASE NETRAL (NPr) TERMOSTABIL DARI *Geobacillus* sp. DENGAN SEL INANG *Escherichia coli* DH5α

INTISARI

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Enzim protease netral termostabil dihasilkan oleh bakteri termofilik yang dapat tumbuh optimal pada kondisi lingkungan yang bersuhu tinggi. Enzim protease yang dihasilkan oleh bakteri ini sangat bermanfaat dalam bidang industri seperti: deterjen, kulit, tekstil, makanan maupun obat-obatan. *Geobacillus* sp. DS3 merupakan bakteri termofilik yang tumbuh optimal pada suhu 55ºC – 65ºC dan bersifat neutrofilik yang dapat tumbuh optimal pada lingkungan dengan pH 6,2 – 7,5. Tujuan dari penelitian ini yakni kloning gen penyandi protease netral (NPr) termostabil yang diproduksi oleh *Geobacillus* sp. DS3 termofilik. Tahapan penelitian yang dilakukan adalah kloning protease netral (NPr) termostabil dari *Geobacillus* sp. DS3 ke plasmid pETSUMO menggunakan metode kloning TA. Kemudian, plasmid rekombinan ditransformasi menggunakan metode *heat shock* ke sel kompeten *Escherichia coli* DH5α Selanjutnya, dilakukan analisis transforman dengan metode koloni PCR dan *sequencing* untuk mengetahui sekuen dari fragmen DNA dan orientasinya. Selanjutnya, dibuat pohon filogentik untuk mengetahuai kekerabatan antara gen penyandi protease netral, protease asam dan protease basa. Hasil dari penelitian ini adalah bahwa plasmid rekombinan pETSUMO NPr termostabil dapat dikloning dengan *Escherichia coli* DH5α dan gen penyandi NPr dari *Geobacillus* sp. DS3 memiliki kekerabatan paling dekat dengan M4 family metallopeptidase *Geobacillus vulcani.*

Kata kunci: enzim protease netral termostabil, plasmid rekombinan pETSUMO NPr, *Geobacillus* sp. DS3, *Escherichia coli* DH5α

CLONING OF GENE THAT ENCODING THERMOSTABLE NEUTRAL PROTEASE (NPr) FROM *Geobacillus* SP. DS3 TO *Escherichia coli* DH5α

ABSTRACT

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Thermostable neutral protease enzymes are produced by thermophilic bacteria that can grow optimally in high-temperature environmental conditions. Protease enzymes produced by these bacteria are very useful in industrial fields such as detergents, leather, textiles, food, and medicine. *Geobacillus* sp. DS3 is a thermophilic bacteria that grows optimally at a temperature of 55ºC - 65ºC and is neutrophilic which can grow optimally in an environment with a pH of 6.2 - 7.5. The purpose of this study was to clone the gene encoding thermostable neutral protease (NPr) produced by *Geobacillus* sp. DS3. The stages of the research carried out were cloning thermostable neutral protease (NPr) from *Geobacillus* sp. DS3 to the pETSUMO plasmid using the TA cloning method. Then, the recombinant plasmid was transformed using the heat shock method into competent cells of *Escherichia coli* DH5α. Furthermore, transformant analysis was carried out using the colony PCR method and sequencing to determine the sequence of DNA fragments and their orientation. Furthermore, a phylogenetic tree was made to determine the kinship between the genes encoding neutral protease, acid protease, and basae protease. The results of this study are that the thermostable pETSUMO NPr recombinant plasmid can be cloned with *Escherichia coli* DH5α and the NPr-encoding gene from *Geobacillus* sp. DS3 has the closest kinship to the NPr-encoding gene from *Bacillus subtilis* protease. The results of this research are that the thermostable pETSUMO NPr recombinant plasmid can be cloned with *Escherichia coli* DH5α and the gene encoding NPr from *Geobacillus* sp. DS3 is the closest relative to M4 family metallopeptidase *Geobacillus vulcani.*

Keywords: thermostable neutral protease enzyme, recombinant plasmid pETSUMO NPr, *Geobacillus* sp. DS3, *Escherichia coli* DH5α