

**PEMODELAN STRUKTUR PROTEASE NETRAL (NPr)  
TERMOSTABIL DARI *Geobacillus* sp. DAN ANALISIS  
PENGHAMBATAN OLEH PHOSPHORAMIDON**

**INTISARI**

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Protease merupakan enzim yang mampu mengkatalisis pemotongan ikatan peptida pada suatu protein menjadi asam amino dan peptida pendek. Protease digunakan dalam industri pangan untuk pengempukan daging, produksi hidrolisat protein, keju, roti, maupun bir. *Geobacillus* sp. yang diisolasi dari kawah Sikidang, Dieng, Jawa Tengah dapat tumbuh optimum pada suhu 70°C dan diketahui memiliki aktivitas proteolitik. Penelitian sebelumnya telah dilakukan amplifikasi *open reading frame* (ORF) protease netral termostabil (NPr) yang berukuran 1.639 bp. Pada penelitian ini, dilakukan pemodelan struktur tiga dimensi enzim NPr dari sekuens ORF NPr *Geobacillus* sp. dengan metode *homology modelling*. Analisis penghambatan oleh phosphoramidon yang merupakan inhibitor alami protease dilakukan dengan metode *Molecular Docking*. Hasil *homology modelling* didapatkan struktur NPr yang memiliki homologi yang tinggi dengan thermolysin M04.001. Struktur tiga dimensi NPr yang dimodelkan terdiri dari domain Peptidase M4 dan Peptidase M4 C-terminal pada urutan residu ke-235 sampai ke-528. Domain ini diketahui memiliki fungsi metalloendopeptidase. Motif pengikatan ion zink pada model NPr diprediksikan sebagai motif 'HEALTH' dengan *catalytic triad* Glu139, His 142 dan His227. Hasil penambatan menunjukkan adanya ikatan hidrogen terkuat sebesar 2,4 Å antara oksigen *double bond* gugus phosphoramidate pada phosphoramidon dengan residu Trp111 pada NPr. Metalloprotease mengikat ion metal seperti  $Zn^{2+}$  pada sisi aktif. Terdapat interaksi antara phosphoramidon dengan ion zink NPr sehingga menyebabkan penghambatan aktivitas NPr. Residu katalitik NPr His142, Glu162, dan His 227 juga dihambat oleh phosphoramidon. Dengan demikian, phosphoramidon diduga termasuk inhibitor kompetitif. Perlu dilakukan *X-ray crystallography* penghambatan phosphoramidon pada NPr.

**Kata kunci: protease netral termostabil (NPr), *Geobacillus* sp., *Homology Modelling*, *Molecular Docking*, inhibitor phosphoramidon**

**MODELLING OF THERMOSTABLE NEUTRAL PROTEASE (NPr)  
STRUCTURE OF *Geobacillus* sp. AND INHIBITION ANALYSIS OF  
PHOSPHORAMIDON**

**ABSTRACT**

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Protease is an enzyme which catalyzes the cleavage of protein peptide bonds into short peptides and amino acids. Proteases are used in the food industry for meat tenderization, protein hydrolysate production, cheese, bread, and beer. *Geobacillus* sp. isolated from Sikidang crater, Dieng, Central Java can grow optimally at 70°C. It is also known to have proteolytic activity. Previous study has carried out amplification of the thermostable neutral protease (NPr) open reading frame (ORF) measuring 1,639 bp. In this study, the three-dimensional structure of NPr enzyme was modeled from the *Geobacillus* sp. ORF NPr sequence using Homology Modelling method. Analysis of inhibition by phosphoramidon, a natural protease inhibitor, was carried out by Molecular Docking method. The modelling resulted in NPr structure with high similarity with thermolysin M04.001. The three-dimensional structure of the modeled NPr consists of Peptidase M4 and C-terminal Peptidase M4 domains at sequence residue 235 to 528. These domains are known to have metalloendopeptidase function. The zinc binding motif of NPr is predicted to be the 'HEALTH' motif with catalytic triad of Glu139, His 142, and His227. The docking showed the strongest hydrogen bond of 2,4 Å between oxygen double bond of the phosphoramidate group on the phosphoramidon and the Trp111 residue of NPr. Metalloproteases bind metal ions such as  $Zn^{2+}$  at the active site of the enzyme. Interaction between phosphoramidon and the zinc ion of NPr is also detected, which then causes inhibition of NPr activity. Phosphoramidon also inhibits some catalytic residues of NPr such as His142, Glu162, and His 227. Thus, phosphoramidon is suspected to be a competitive inhibitor. It is necessary to perform X-ray crystallography on inhibition of phosphoramidon on NPr.

**Keyword: thermostable neutral protease, *Geobacillus* sp., Homology  
Modelling, Molecular Docking, phosphoramidon inhibitor**