BIOTEKNOLOGI

LAPORAN HASIL
HIBAH PENELITIAN TIM PASCASARJANA
(HIBAH PASCA)

PENINGKATAN AKTIVITAS ENZIM HEMISELULASE DENGAN REKAYASA PROTEIN UNTUK PENGOLAHAN BIOMASSA BERBASIS LIGNOSELULOSA

Prof. Dr. Ni Nyoman Tri Puspaningsih, M.Si.
Prof. Dr. Eddy Bagus W., M.S., Sp.MK.
A.A. Istri Ratnadewi
Ratna Melinda
M. Fanani

Dibiayai oleh DIPA Universitas Airlangga, sesuai dengan Surat Keputusan Rektor tentang Kegiatan Penelitian Multi Tahun, Pengabdian kepada Masyarakat Mono Tahun, dan Pengabdian kepada Masyarakat Multi Tahun Universitas Airlangga Tahun Anggaran 2012 Nomor : 2613/H3/KR/2012, Tanggal 9 Maret 2012

UNIVERSITAS AIRLANGGA
2012

ABSTRACT

SUBSTRATE SPECIFICITY OF α-L-ARABINOFURANOSIDASE GH51 FROM *Geobacillus thermoleovorans* IT-08 TOWARD *Mycobacteria* CELL WALL

(hasil tesis S2 atas nama M. Fanani)

The purpose of this research is to investigate the substrate stereospecificity of α-L-arabinofuranosidase GH51 *Geobacillus thermoleovorans* IT-08 (AbfA) toward *Mycobacteria* cell wall. In general, there are 3 stages in this research are (1) the production of AbfA recombinant from *E. coli* BL21(*DE3*)/pET-abfA, (2) specific activity assay of AbfA recombinant, (3) substrate stereospecificity analysis of AbfA in silico. In laboratory methods, AbfA has hydrolase activity toward D-arabinofuranoside (H37Rv and BFCC). However its activity toward L-arabinofuranoside (arabinogalactan, pectin, oat spelt xylan and arabinan) higher than its hydrolase activity toward D-arabinofuranoside (H37Rv and BFCC). On silico analysis, hydrolase activity AbfA toward D-arabinofuranoside may occur due to fingerprint interactions between the ligand and catalytic residue Glu294. Based on in silico analysis, the catalytic mechanism of AbfA toward D-arabinofuranoside was suggested following model catalytic mechanism of α-L-arabinofuranosidase GH51 toward L-arabinofuranoside substrate. Effect of steric hindrance by Trp99 and Trp298 at the sub-site -1 rationalize the substrate specificity toward D-arabinofuranoside lower than L-arabinofuranoside. This thesis research was the first reported that AbfA has catalytic activity toward D-Arabinofuranoside derived from Mycobacteria cell wall.

Key words : α-L-arabinofuranosidase GH51, substrate stereospecificity, fingerprint interaction, steric hydrance.

ABSTRACT

Characterization of α-L-arabinofuranosidase (AbfA) Variant Biochemical Properties and In Silico Study on The Effect of Mutation to Its Structure

(hasil tesis atas nama Ratna Melinda)

Geobacillus thermoleovorans IT-08 α-L-arabinofuranosidase (AbfA) is an enzyme with optimum activity around neutral pH (6-8). Its variant (variant A9) exhibit an increased activity at pH 9. In this study, a nucleotide alignment to the wildtype (DQ387046.1) was done to determine the modification in *abfa* variant A9 gene. The enzyme expressed in *E. coli* BL21 (*DE3*)/pBM5abf were characterized for its biochemical properties. Protein tertiary structure model which built by aligning the sequence to *Geobacillus stearothermophilus* T-6 α-L-arabinofuranosidase protein crystal structure (PDB accession number: 1PZ3), was subjected to determine the structure alteration. The relation between change in biochemical property and structure alteration was also investigated. Substitution at three bases in *abfa* variant A9 gene (A137G; T615A; A853G) resulted in amino acids replacement (Gln46Arg; Asp205Glu; Lys285Glu). Expression of *abfa* variant gene was detected using SDS PAGE, shown a 61 KDa band corresponds to AbfA variant A9. The partially purified enzyme displayed optimum activity at pH 7 and 70$_o$C. AbfA variant A9 was stable for

24 hours at pH 6-9 (in 4oC) and lost almost 70% of its activity on 16 hours incubation at 70oC. Superimpose of Abfa variant with the wildtype tertiary structure model shown RMSD value 0.05%. In silico analysis of non covalent interaction revealed that non covalent interactions within the protein were reduced due to amino acid replacement. Substitution of Arg46 to Gln46 (ΔΔG: +0,22) decrease 3 Van der Waals interaction, Asp205Glu substitution (ΔΔG: +2.32) resulted in decrease of 3 Hydrogen bonds and 1 Van der Waals bond and generation of 1 electrostatic interaction, substitution of Lys285 by Glu (ΔΔG: -0.09) decreased 1 Van der Waals interaction but reduced the repulsive effect arises from positive side chains around residue 285. The decrease in AbfA variant thermostability was related to the reduction of some non covalent interactions especially Hydrogen bonds and Van der Waals interaction, because of changes in structure.

Keywords: α-L-arabinofuranosidase (AbfA), mutation, biochemical properties
protein tertiary structure model, non covalent interaction