

ABSTRAK

KARAKTERISASI GEN AKTINOBAKTERI LAUT SEBAGAI PENGHASIL ENZIM KITINASE SECARA *SOLID STATE* *FERMENTATION* DARI SUBSTRAT KULIT UDANG

Oleh

WAWAN ABDULLAH SETIAWAN

Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi spesies aktinobakteri laut penghasil enzim kitinase melalui analisis filogenetik serta mengkarakterisasi gen aktinobakteri laut sebagai penghasil enzim kitinase melalui fermentasi padat (*Solid State Fermentation/SSF*) dari substrat kulit udang. Isolat aktinobakteri sebanyak 6 isolat dari laut Bali dan Gorontalo diisolasi menggunakan media selektif *International Streptomyces Project 2* (ISP 2) dan koloid kitin. Identifikasi spesies dilakukan melalui pengamatan morfologi menggunakan mikroskop cahaya dan *Scanning Electron Microscope* (SEM) serta analisis filogenetik berbasis sekuen gen 16S rRNA, menghasilkan spesies *Pseudonocardia carboxydvorans* 18A13O1, *Pseudonocardia antitumoralis* 18D36-A1, *Micrococcus unila* 18D36-A2, *Brevibacterium linens* 19A07-A1, *Streptomyces tritolerans* 19B19A1, dan *Kocuria palustris* 19C38A1. Pengujian aktivitas kitinolitik menunjukkan *Streptomyces tritolerans* 19B19A1 memiliki aktivitas tertinggi dengan indeks kitinase 0,324. Karakterisasi gen kitinase pada isolat ini meliputi ekstraksi RNA, sintesis cDNA, amplifikasi PCR dengan primer ChiA, sekuensing, dan analisis *in silico* melalui ORF finder, BLAST, pemodelan struktur protein, serta molecular docking dengan kitin kulit udang. Hasil menunjukkan gen kitinase *S. tritolerans* 19B19A1 berukuran 882 nukleotida yang mengkodekan 293 asam amino. Gen yang didapat menunjukkan homologi 96,61 % dengan enzim kitinase *Streptomyces* di GenBank dengan kode akses WP_011029996.1. Analisis docking molekuler menggunakan AutoDock mengonfirmasi interaksi kuat antara enzim kitinase dan substrat kitin. Nilai *free energy binding* (FEB) sebesar -3,93 dan konstanta inhibisi (KI) sebesar 1320. Nilai FEB dan KI tersebut lebih rendah dari pembandingnya 3EBV yang sudah terdeposit di Protein Data Bank (PDB) yaitu -3,83 dan 1380. Penelitian ini memberikan kontribusi pada pengembangan rekayasa

genetik untuk produksi enzim kitinase dan pemanfaatan limbah kulit udang secara berkelanjutan.

Kata Kunci : aktinobakteri laut, enzim kitinase, fermentasi padat, kulit udang, karakterisasi gen

ABSTRACT

CHARACTERIZATION OF MARINE ACTINOBACTERIAL GENE AS CHITINASE ENZYME PRODUCER THROUGH SOLID STATE FERMENTATION FROM SHRIMP SHELL SUBSTRATE

By

WAWAN ABDULLAH SETIAWAN

This study aims to isolate and identify marine actinobacterial species producing chitinase enzymes through phylogenetic analysis and to characterize the chitinase-producing genes of marine actinobacteria through solid-state fermentation (SSF) using shrimp shell substrate. Six actinobacterial isolates from the seas of Bali and Gorontalo were isolated using selective International Streptomyces Project 2 (ISP 2) medium and colloidal chitin. Species identification was conducted through morphological observation using light microscopy and Scanning Electron Microscopy (SEM), as well as phylogenetic analysis based on 16S rRNA gene sequences, resulting in the identification of *Pseudonocardia carboxydivorans* 18A13O1, *Pseudonocardia antitumoralis* 18D36-A1, *Micrococcus unila* 18D36-A2, *Brevibacterium linens* 19A07-A1, *Streptomyces tritolerans* 19B19A1, and *Kocuria palustris* 19C38A1. Chitinolytic activity testing revealed that *Streptomyces tritolerans* 19B19A1 exhibited the highest activity with a chitinase index of 0.324. Characterization of the chitinase gene in this isolate involved RNA extraction, cDNA synthesis, PCR amplification using ChiA primers, sequencing, and in silico analysis using ORF Finder, BLAST, protein structure modeling, and molecular docking with shrimp shell chitin. The results showed that the chitinase gene of *S. tritolerans* 19B19A1 consists of 882 nucleotides encoding 293 amino acids. The obtained gene exhibited 96.61% homology with the chitinase enzyme of *Streptomyces* in GenBank under accession code WP_011029996.1. Molecular docking analysis using AutoDock confirmed a strong interaction between the chitinase enzyme and the chitin substrate, with a free energy binding (FEB) value of -3.93 and an inhibition constant (KI) of 1320. These FEB and KI values were lower than those of the reference 3EBV deposited in the Protein Data Bank (PDB), which were -3.83 and 1380, respectively. This study contributes to the development of genetic engineering for chitinase enzyme production and the sustainable utilization of shrimp shell waste.

Keywords: marine actinobacteria, chitinase enzyme, solid-state fermentation, shrimp shell, gene characterization