ABSTRACT

MOLECULAR CHARACTERIZATION OF LIPASE GENE FROM *Alcaligenes* sp. JG3 BACTERIUM

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Molecular characterization of amplified lipase gene from *Alcaligenes* sp. JG3 has been accomplished. This study is aimed to confirm the taxonomy status of strain JG3, to amplify its lipase gene, to characterize its conserved and catalytic site regions, and to model its 3-D structure form.

The bacterial taxonomic confirmation was carried out using 16S rRNA gene analysis. The lipase gene was amplified using PCR with a primer pair designed based on lipase nucleotide sequence from *Alcaligenes faecalis* subsp. *faecalis* NCIB 8687, which has been published in gene bank. The amplified sequences were aligned with others lipase genes from database to know its conserved and catalytic regions. Moreover, the *in silico* tools, Conseq and I-TASSER server, were employed to model and analyze the structural properties of the obtained protein sequence.

The 16S rRNA gene analysis confirmed that strain JG3 belongs to *Alcaligenes* genus by 96 % identity. PCR amplification resulted in 1067 bp of lipase DNA sequence (LipJG3). This sequence shares 91% and 98% identity towards lipase gene from *A. faecalis* in nucleotide and deduced amino acid level respectively. Amino acid sequence and structural analysis employing *in silico* tools revealed that the LipJG3 belongs to ABC (ATP-binding cassette) transporter protein and contained three highly conserved region consisting of EASGSKT, VILLD, and LSGGQQQRVAIA known as ATP-binding signature at Walker-A and Walker-B motifs and the S signature of ABC transporter family respectively. In addition, its 3-D structure has been suggested but the role of these conserved sequences have not been fully understood as hydrolyzing enzyme.

Keywords: *Alcaligenes,* lipase gene, LipJG3

INTISARI

KARAKTERISASI MOLEKULAR GEN LIPASE DARI BAKTERI *Alcaligenes* sp. JG3

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Telah dilakukan karakterisasi secara molekular terhadap lipase gen teramplifikasi dari bakteri *Alcaligenes* sp. JG3. Penelitian ini bertujuan untuk mengkonfirmasi posisi taksonomi dari strain JG3, mengamplifikasi gen lipase, mengkarakterisasi daerah lestari dan sisi katalitiknya, serta memodelkan struktur 3D.

Konfirmasi taksonomi bakteri dilakukan dengan analisis gen 16S rRNA. Gen lipase diamplifikasi menggunakan PCR dengan pasangan primer yang didesain berdasarkan urutan gen lipase dari *Alcaligenes faecalis* subsp. *faecalis* NCIB 8687 yang telah terpublikasi di *genebank*. Sekuen teramplifikasi disejajarkan dengan gen lipase lain yang ada di *database* untuk mengetahui daerah lestari dan sisi katalitik. Perangkat lunak *online* seperti Conseq dan I-TASSER digunakan untuk pemodelan dan analisis sifat dari struktur protein yang diperoleh.

Berdasarkan analisis gen 16S rRNA, strain JG3 digolongkan ke dalam genus *Alcaligenes* dengan kemiripan 96%. Amplifikasi PCR menghasilkan lipase gene (LipJG3) 1067 pb. Urutan DNA ini secara beturutan menunjukkan kemiripan 91% dan 98% pada tingkat nukleotida dan asam amino. Analisis urutan asam amino dan strukturnya secara *in silico* diketahui bahwa LipJG3 tergolong dalam golongan protein ABC (ATP-*binding cassette*) transporter dan memiliki tiga urutan asam amino yang lestari, yaitu EASGSKT, VILLD, dan LSGGQQQRVAIA dimana ketiganya dikenal sebagai sisi ikat (pengenalan) ligan ATP pada motif *Walker-A* dan *Walker-B* serta S *signature* dari golongan protein ABC transporter. Berdasarkan homologi tersebut, struktur 3-D dari LipJG3 telah diusulkan, namun belum dapat dimengerti bagaimana fungsi dan peran dari urutan-urutan lestari tersebut sebagai enzim penghidrolisis.

Kata Kunci: *Alcaligenes*, lipase gen, LipJG3