Intisari

Kitinase merupakan enzim yang dapat mendegradasi kitin. Kitinase dapat diperoleh dari bakteri kitinolitik *E. cloacae.* Tujuan dari penelitian ini adalah untuk melakukan kloning gen kitinase dari isolat TB 22 (*E. cloacae*) bekteri kitinolitik serta mengetahui ekspresi gen dari isolat TB 22 (*E. cloacae*). Kloning gen dilakukan dengan melakukan restriksi pada gen target dan vektor PET28a menggunakan enzim restriksi BamHI dan EcoRI. Hasil restriksi dari gen dan vektor kemudian digabungkan atau ligasi. Transformasi DNA rekombinan kedalam *host* bakteri (*E.coli* DH5α) menggunakan metode *heat shock.* Konfirmasi gen kitinase dilakukan dengan melakukan PCR koloni. Koloni yang positif membawa gen kitinase selanjutnya diisolasi untuk mendapatkan plasmid rekombinan. Plasmid rekombinan selanjutnya disekuensing untuk mengetahui urutan basa nitrogennya. Hasil kloning menunjukkan kitinase rekombinan memiliki berat molekul 1.290 bp yang. Analisis sekuen menggunakan program BLAST menunjukkan bahwa plasmid rekombinan memiliki kesamaan dengan kitinase dari spesies spesies *E. cloacae* CAV1669, *E. cloacae* strain CAV1668, *E. cloacae* CAV1411, *E. cloacae* strain CAV1311, *E. cloacae* strain ECNIH2 masing-masing sebesar 99%. Ekspresi gen dilakukan dengan transformasi kitinase rekombinan kedalam *E. coli* BL21. Koloni yang tumbuh dari hasil transformasi *E. coli* BL21 ditumbuhkan kedalam medium LB cair dan diinduksi dengan IPTG (Isoprophyl-1-thio-β-D-galactopyranoside) yang kemudian dikultur selama 24 jam. Kultur bakteri dipanen dan dilakukan lisis menggunakan panas dan selanjutnya di*running* kedalam SDS PAG*E.* Ekspresi gen menunjukkan bahwa ukuran protein sebesar 45,7 kDa.

Kata kunci : *E. cloacae*, ekspresi gen, kitinase, kloning, kitinase rekombinan

Abstract

Chitinase is an enzyme that degrades chitin. Chitinase can be obtained from a chitinolytic bacterium *E. cloacae*. The aims of this research were to clone chitinase gene from *E. cloacae* TB 22. Gene cloning was done by restriction enzim of BamHI and ECoRI and cloned to PET28a vector. Transformation of DNA recombinant into the bacterial host (*E. coli* DH5α) used heat shock method. Confirmation of the chitinase gene was done by colony PCR. Positive colonies were isolated to obtain recombinant plasmid. Then, the recombinant plasmid were sequenced to determine the sequence of bases. The cloning results showed that the molecular weight of recombinant chitinase gene was 1.290 bp. Sequence analysis using BLAST program indicated that the chitinase gen was highly similar to chitinase from species spesies *E. cloacae* CAV1669, *E. cloacae* strain CAV1668, *E. cloacae* CAV1411, *E. cloacae* strain CAV1311, *E. cloacae* strain ECNIH2 with 99% similary. Gene expression was done by recombinant chitinase transformation into *E.* coli BL21. Colonies that grew from the transformation of *E.* coli BL21 was cultured in liquid LB medium and it was induced with IPTG (Isoprophyl-1-thio-β- D-galactopyranoside) for 24 hours. The bacterial culture was harvested and lysed, then the protein was subjected to SDS-PAGE. Resulting 45,7 kDa of protein.

Keywords: Chitinase, cloning, *E. cloacae*, gene expression, recombinant chitinase