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Isolasi gen fungsional resistan eritromisin dan kanamisin dari bacillus halodurans CM1 = Isolation of erythromycin and kanamycin resistance functional gene from bacillus halodurans CM1

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Abstrak

Bacillus halodurans CM1 berpotensi sebagai inang dalam menghasilkan beberapa jenis enzim yang berguna, seperti xilanase, lipase, dan protease. Selain sebagai penghasil enzim, salah satu gen potensial lainnya adalah gen resistan antibiotik. Penelitian ini bertujuan untuk mengetahui kemampuan resistansi B. halodurans CM1 terhadap eritromisin dan kanamisin serta diperoleh produk gen fungsional resistan eritromisin dan kanamisin dari B. halodurans CM1. Isolasi gen eritromisin dan kanamisin dari B. halodurans CM1 belum pernah dilakukan. Berdasarkan uji KHM, B. halodurans CM1 resistan terhadap eritromisin dan kanamisin. Isolasi gen resistan eritromisin dan kanamisin dilakukan dengan menggunakan metode amplifikasi PCR. Produk PCR yang diduga gen resistan eritromisin, yaitu gen ErmK dan BH0381. Produk PCR yang diduga gen resistan kanamisin, yaitu gen aadK dan APH. Gen ErmK dikloning dengan menggunakan kloning vektor pGEM-T Easy, sedangkan gen BH0381, aadK, dan APH dikloning dengan menggunakan kloning vektor pJET1.2/blunting. Vektor rekombinan ditransformasi ke Escherichia coli DH5alfa. Hasil analisis sekuens DNA menggunakan BLAST menunjukkan bahwa gen ErmKCM1 dan BH0381 masing-masing memiliki kemiripan 99,65% (GenBank No access: BH0380, ErmK) dan 99,45% (GenBank No access: BH0381, mphB) dengan sekuens dari B. halodurans C-125. Sekuens gen ErmKCM1 dan BH0381CM1 menunjukkan bahwa dua gen tersebut merupakan gen yang fungsional. Sekuens upstream dari gen BH0381CM1 dianalisis dan diperoleh bahwa terdapat 50 pb yang diduga promoter. Hasil analisis sekuens DNA menggunakan BLAST menunjukkan bahwa gen aadKCM1 dan APHCM1 masing-masing memiliki kemiripan 97,73% (GenBank No access: BH0322) dan 99,47% (GenBank No access: BH0326) dengan sekuens dari B. halodurans C-125. Hasil penyejajaran gen aadKCM1 menunjukkan adanya delesi enam pasang nukleotida pada lokus ke-354 hingga 359 yang menyebabkan frameshift, sehingga gen aadKCM1 tidak memiliki sekuens yang open reading frame (ORF). Hasil translasi gen aadKCM1 dan APHCM1 menunjukkan bahwa hanya sekuens gen APHCM1 dapat ditranslasi menjadi protein yang fungsional. Hasil uji resistansi kanamisin pada transforman yang membawa plasmid rekombinan promoter gen APHCM1-ORF dapat menyandikan resistan kanamisin dengan konsentrasi kanamisin sebesar 20 µg/mL.
.....Bacillus halodurans CM1 has potential as a host in producing several types of useful enzymes, such as xylanase, lipase, and protease. Besides industrial enzymes, one of the other potential genes is the antibiotic-resistant gene. This study aims to determine the ability of B. halodurans CM1 resistance to erythromycin and kanamycin and to obtain erythromycin and kanamycin-resistant functional gene products from B. halodurans CM1. Isolation of erythromycin and kanamycin genes from B. halodurans CM1 has never been done. Based on the MIC test, B. halodurans CM1 is resistant to erythromycin and kanamycin. Erythromycin and kanamycin resistance gene isolation was carried out using PCR amplification method. PCR products suspected of being erythromycin resistance genes, namely ErmK and BH0381 genes. PCR products suspected of being kanamycin resistance genes, namely genes aadK and APH. The ErmK gene was cloned using the pGEM-T Easy vector cloning, while the BH0381, aadK, and APH genes were cloned using the

pJET1.2/blunting vector cloning. The recombinant vector was transformed into Escherichia coli DH5ï?¡. The results of DNA sequence analysis using BLAST showed that the ErmKCM1 and BH0381 genes each had 99.65% similarities (GenBank No access: BH0380, ErmK) and 99.45% (GenBank No access: BH0381, mphB) with the sequence of B. halodurans C-125. The results of the translation of the ErmKCM1 and BH0381CM1 genes indicate that the two genes are functional genes. The upstream sequence of the BH0381CM1 gene was analyzed and it was found that 50 bp was suspected as a promoter. The results of DNA sequence analysis using BLAST showed that the aadKCM1 and APHCM1 genes each had a similarity of 97.73% (GenBank No access: BH0322) and 99.47% (GenBank No access: BH0326) with the sequence of B. halodurans C-125. The alignment of the aadKCM1 gene shows the deletion of six nucleotide pairs at the 354-359 locus that causes frameshift, so the aadKCM1 gene does not have an open reading frame (ORF) sequence. The translation of aadKCM1 and APHCM1 genes show that only the APHCM1 gene sequence can be translated into a functional protein. The results of kanamycin resistance test on transformants carrying plasmids with native promoter APHCM1-ORF gene can encode kanamycin resistance with a kanamycin concentration of 20 µg/mL.