Transformasi plasmid rekombinan yang termetilasi sebagai pembawa kaset gen protease untuk proses integrasi kromosomal ke bacillus halodurans CM1 = Transformation of methylated plasmid as protease gene carriers for the chromosomal integration into bacillus halodurans CM1

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Abstrak

Efisiensi transformasi plasmid rekombinanyang rendah ke dalam bakteri wild-type disebabkan oleh mekanisme pertahanan dari sel bakteri. Enzim restriksi dalam sel bakteri target dapat mendegradasi plasmid rekombinan. Transformasi plasmid pBBRE194 rekombinan yang mengandung gen protease dari Bacillus halodurans CM1 (pBBRE194 prot-CM1) telah dilakukan, tetapi efisiensi transformasi rendah dan klona rekombinan yang didapatkan tidak menunjukkan sifat yang stabil. Penelitian ini bertujuan untuk memetilasi plasmid pBBRE194 prot-CM1 dan transformasi plasmid pBBRE194 prot-CM1 termetilasi secara konjugasi ke B. halodurans CM1, kemudian menganalisis aktivitas enzim protease yang dihasilkan B. halodurans CM1 pembawa plasmid pBBRE194 prot-CM1. Aktivitas spesifik (U/mg) protease yang dihasilkan oleh B. halodurans CM1 rekombinan dianalisis dengan cara mengukur unit aktivitas (U/mL) dengan metode Amano dan kadar protein (mg/mL) dengan metode Bradford. Plasmid pBBRE194 prot-CM1 dalam E. coli TOP10 berhasil dimetilasi oleh gen methylase pada plasmid pPAMC125 yang diinduksi oleh 0,02% L-arabinosa. Konjugasi plasmid pBBRE194 prot-CM1 termetilasi ke B. halodurans CM1 berhasil dilakukan dan terpilih 1 klona B. halodurans CM1 rekombinan yang telah terverifikasi. Verifikasi dilakukan berdasarkan kemampuan klona dalam mendegradasi protein pada media selektif yang mengandung skim milk dan tetracycline. Verifikasi berdasarkan polymerase chain reaction dengan mendeteksi sekuens gen resistan tetracycline pada klona juga dilakukan. Proses PCR koloni pada B. halodurans CM1 rekombinan menunjukkan terbentuknya pita DNA ukuran 1024 bp yaitu ukuran gen resistan tetracycline pada plasmid pBBRE194 prot-CM1. Hasil analisis menunjukkan bahwa aktivitas spesifik B. halodurans CM1 rekombinan (660,700 U/mg) lebih rendah dibandingkan dengan kontrol negatif, yaitu B. halodurans CM1 wild-type (1054,928 U/mg). Analisis aktivitas protease dilakukan pada suhu 50 oC dan pH 12.
.....Transformation rate into wild-type bacteria is commonly low because of the cell defense mechanism of the bacteria. Restriction modification (RM) in bacteria cells can prevent the introduction of recombinant plasmid into target bacteria. Previously, the transformation of recombinant shuttle vector pBBRE194 containing protease gene (pBBRE194 prot-CM1) into wild-type Bacillus halodurans CM1 has been conducted. However, the transformation rate seemed low, and the stable recombinant clones could not be obtained. Therefore, in vivo methylation of this plasmid in E. coli has to be done before genetic transformation into the wild-type bacterium, to obtain stable recombinant B. halodurans CM1. In this study, a plasmid with artificial modification (pPAMC125) harboring genes encoding for the modification enzymes (methylases) from another strain, B. halodurans C-125, and pBBRE194 prot-CM1 plasmid were transformed by conjugation into B. halodurans CM1. Specific activity (U/mg) of protease produced by recombinant B. halodurans CM1 was analyzed by measuring activity units (U/mL) by the Amano method and protein quantity (mg/mL) by the Bradford method. The pBBRE194 prot-CM1 might be methylated by methylases

that was induced by 0.02% L-arabinose. Conjugation of the methylated pBBRE194 prot-CM1 to B. halodurans CM1 was successfully carried out and recombinant B. halodurans CM1 was verified. The verification of a recombinant clone is based on its ability to degrade proteins on selective media containing skim milk and tetracycline. Also beside, verification based on polymerase chain reaction was also carried out by detecting tetracycline resistance gene sequences. The PCR result in recombinant clone amplified the 1024 bp DNA, which the size of the tetracycline resistance gene in pBBRE194 prot-CM1 plasmid. The analysis of protease activity showed that the specific activity of the recombinant clone (660.700 U/mg) was lower than the negative control, which B. halodurans CM1 wild-type (1054.928 U/mg). The analysis was carried out at 50oC and pH 12.