Pengklonaan gen Beta Defensin 30 Mencit Untuk Pengkajian Perannya Pada Proses Pematangan Spermatozoa di Epididimis = Cloning of Mouse Beta Defensin 30 Gene for Its Role in Epididymal Sperm Maturation

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Abstrak

Latar belakang: Beta defensin diekspresikan terutama oleh sel epitel pada permukaan mukosa berbagai organ seperti kulit, usus, mulut dan saluran genital. Studi sebelumnya menunjukkan bahwa beta defensin 30 (Defb30) terekspresi spesifik di epididimis. Defb30 merupakan peptida kationik berukuran kecil yang diduga berperan penting pada proses pematangan spermatozoa di epididimis dan juga berperan sebagai pertahanan host terhadap infeksi mikroba. Untuk mempelajari aktivitas antimikroba Defb30 ini diperlukan analisis pada tingkat protein dan hal tersebut memerlukan protein dalam jumlah yang cukup. Karena itu perlu dilakukan suatu rekayasa genetika untuk pembuatan protein rekombinan DEFB30.
Metode: Gen sintetik penyandi protein DEFB30 yang telah dioptimasi kodonnya diklona ke dalam vektor pQE-80L, suatu plasmid yang mengandung sistem ekspresi untuk prokariota. Plasmid rekombinan yang mengandung sisipan gen target dikonfirmasi dengan analisis enzim restriksi dan sekuensing. Selanjutnya plasmid rekombinan di ekpresikan ke dalam E. coli BL21 dan diinduksi menggunakan IPTG (Isopropyl-1-Thio-d-Galactopyranoside) dengan berbagai waktu inkubasi. Deteksi protein rekombinan dilakukan dengan SDS-PAGE dan westernblotting. IMAC (Immobilized Metal Affinity Chromatography) digunakan untuk mempurifikasi protein rekombinan. Uji antimikroba protein rekombinan dilakukan dengan cara pengukuran nilai optical density (OD) dan dianalisis hasilnya menggunakan uji one way anova.
Hasil: Gen sintetik penyandi protein rekombinan DEFB30 berhasil dikonstruksi pada plasmid pQE-80L. Ekspresi ke dalam E. coli BL21 menghasilkan suatu protein fusi setelah diinduksi menggunakan IPTG selama 4 jam. Hasil analisis protein rekombinan dengan westernblotting menggunakan antibodi Anti-His G-HRP menunjukkan terbentuk pita tebal yang berukuran diatas 10 kDa (±12 kDa). Uji antimikroba protein rekombinan DEFB30 menunjukkan bahwa DEFB30 dapat menghambat pertumbuhan bakteri Eschericia coli dan Bacillus subtilis.
Kesimpulan: Gen sintetik penyandi beta defensin 30 berhasil diklona ke dalam plasmid pQE-80L. Ekspresikan protein rekombinan DEFB30 menghasilkan suatu protein fusi berukuran ±12kDa. Protein rekombinan DEFB30 terbukti memiliki sifat antimikroba terhadap Eschericia coli dan Bacillus subtilis.
.....ackground: Beta defensins are primarily expressed by epithelial cells at mucosal surfaces, such as those in skin, gut, mouth and genital tracts. Previous studies have demonstrated that beta defensin 30 (Defb30) is exclusively expressed in the epididymis. Defb30 is known as a small cationic antimicrobial peptide which plays an important role in epididymal sperm maturation and also acts as a host defence against microbial infection. Study of Defb30 role in the antimicrobial activity requires generating DEFB30 protein for characterization. For the purpose of this study, genetic engineering was done for the manufacture of the DEFB30 recombinant protein.
Methods: In this study, according to the preferred codon in E. coli, the Defb30 gene was optimized and synthesized. The gene was cloned into pQE-80L vector and subsequently expressed in E. coli BL21; using

IPTG (Isopropyl-1-Thio-d-Galactopyranoside) as an inducer. Detection of recombinant protein was carried out by using SDS-PAGE and westernblotting. IMAC (Immobilized Metal Affinity Chromatography) was used to purify recombinant protein. Optical density measurement was used to analyze antimicrobial property of the DEFB30 recombinant protein.

Results: The synthetic gene was successfully constructed into pQE-80L plasmid and expression of the recombinant protein in E. coli BL21 produced a fusion protein after being induced by IPTG for 4 hours. Westernblotting analysis using Anti-His G-HRP antibody showed band above 10kDa (±12kDa). Antimicrobial assay for DEFB30 recombinant protein showed inhibition towards growth rates of Eschericia coli and Bacillus subtilis.

Conclusion: Defb30 synthetic gene was succesfully cloned into pQE-80L plasmid. Expression of recombinant DEFB30 produced a fusion protein of ±12kDa. This recombinant protein has antimicrobial property towards Eschericia coli and Bacillus subtilis.