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Pengklonaan, ekspresi, dan determinasi sifat antimikroba protein rekombinan SPAG11A mencit untuk mengekplorasi peran beta defensin di epididimis = Cloning expression and antimicrobial determination of mouse recombinant SPAG11A to explore the role of beta defensin in the epididymis

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Abstrak

Spag11a diketahui terekspresi secara spesifik pada kaput epididimis sehingga dimungkinkan protein tersebut memiliki fungsi yang spesifik untuk maturasi spermatozoa. Studi peran SPAG11A dalam maturasi spermatozoa di epididimis memerlukan produksi protein SPAG11A untuk dikarakterisasi. Tujuan dari penelitian ini adalah untuk mengklon, mengekspreesikan, dan mengkarakterisasi sifat antimikroba dari protein rekombinan SPAG11A. Insert cDNA Spag11a yang dihasilkan melalui PCR diklon ke dalam vektor pET100/D-TOPO. Plasmid rekombinan kemudian diekspresikan ke Escherichia coli BL21 DE3 star. Deteksi dari fusi protein rekombinan dilakukan dengan SDS-PAGE dan Western Blotting. IMAC Immobilized Metal Affinity Chromatography digunakan untuk mempurifikasi protein rekombinan. Uji antimikroba protein rekombinan dianalisis melalui pengukuran Optical density.PCR amplifikasi dari cDNA kaput epididimis mencit menghasilkan insert Spag11a berukuran 210bp. Insert tersebut kemudian dikloning ke dalam pET100/D-TOPO menghasilkan 1 rekombinan plasmid dari 10 koloni yang diskrining. Ekspresi rekombinan klon ke dalam E.coli BL21 menghasilkan fusi protein setelah diinduksi IPTG selama 4 jam. Fusi protein dikonfirmasi menggunakan Western Blotting menggunakan antibodi yang mengenali N-terminal His-Tag 21kDa dan protein SPAG11A. Uji antimikroba protein rekombinan SPAG11A mununjukkan tidak ada inhibisi yang signifikan terhadap laju pertumbuhan E.coli dan Bacillus subtilis. Insert Spag11a yang berukuran 210bp berhasil diklon ke dalam vektor pET100/D-TOPO. Ekspresi rekombinan Spag11a menghasilkan fusi protein berukuran 21kDa. Protein rekombinan SPAG11A tidak membawa sifat antimikroba terhadap E.coli dan B. subtilis.

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Spag11a is known to be specifically expressed in the caput region of the epididymis suggesting a specific function for sperm maturation. Study of SPAG11A role in the epididymal sperm maturation requires generating SPAG11A protein for characterization. The objective of this study was to clone, express and characterize antimicrobial property of the recombinant SPAG11A. Spag11a cDNA insert was generated by PCR and cloned in TOPO vector. Recombinant DNA plasmid was subsequently expressed in E coli BL 21 star. Detection of recombinant fusion protein was carried out using SDS PAGE and western immunobloting. IMAC Immobilized Metal Affinity Chromatography was used to purify recombinant protein. Optical density measurement was used to analyse antimicrobial property of the recombinant protein. PCR amplification of mouse caput epididymis cDNA produced a 210 bp insert of Spag11a. Cloning of the insert into TOPO pET100 resulted in 2 recombinants out of 10 colonies that were screened. Expression of recombinant clones in the E coli BL21 produced a fusion protein after being induced IPTG for 4 hours. Fusion protein was confirmed by western immunobloting using two antibodies recognizing N terminal His Tag 21 kDa and SPAG11A protein. Antimicrobial assay for SPAG11A recombinant showed no significant inhibition

towards growth rates of E coli and Bacillus subtilis. A 210 bp Spag11a insert was successfully cloned into TOPO pET100 vector. Expression of recombinant spag11a produced a fusion protein of 21 kDa. SPAG11A recombinant protein does not have antimicrobial property towards E coli and B subtilis.