Ekspresi dan kemampuan inhibitor protease dari protein rekombinan SPINK2 epididimis mencit = Expression and activity of protease inhibitor of mouse epididymal SPINK2 recombinant protein

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Abstrak

Latar belakang: Proses pematangan spermatozoa membutuhkan interaksi antar protein yang disintesis dan disekresikan oleh epitel epdidimis ke lumen di area tertentu. Gen yang terekspresi secara spesifik di region-region tertentu akan menciptakan lingkungan mikro yang kondusif untuk proses pematangan spermatozoa. Spink2 adalah salah satu gen yang diketahui berperan dalam pematangan spermatozoa yang ekspresinya terdekteksi di epididimis. Studi peran SPINK2 membutuhkan protein yang cukup untuk karakterisasi. Tujuan penelitian ini adalah untuk mengklon, mengekspresikan, dan menguji aktivitas protein rekombinan SPINK2.
Metode: Dalam penelitian ini gen mSpink2 dikonstruksikan secara sintetis. Plasmid pQE80L digunakan sebagai vektor ekspresi dan strain sel Escherichia coli yang digunakan adalah Top10 dan BL21. Proses pengklonaan diawali dengan transformasi plasmid pQE80L-mSpink2 ke E. coli Top10 kompeten menggunakan metode heatshock. Proses ekspresi dilakukan dengan penambahan agen induksi Isopropyl-1-Thio-d-Galactopyranoside (IPTG), dipurifikasi dengan kromatografi Ni-NTA. Kemudian dilakukan uji aktivitas protease dengan pembacaan panjang gelombang 660 nm.
Hasil: Plasmid pQE80L-mSpink2 terkonfirmasi berhasil diklon dengan analisis enzim restriksi dan sekuensing. Hasil elekstroforesis menunjukan adanya fragmen DNA dengan panjang 4709 pb dan 258 pb. Hasil ekspresi SDS-PAGE dan western blot menunjukkan SPINK2 berhasil terekspresi pada waktu optimum induksi jam ke-4 dan terdapat pita tebal dengan ukuran 14 kDa. Protein rekombinan SPINK2 berhasil dipurifikasi dan ditunjukan dari hasil western blot pada elusi ke-1 hingga ke-4. Hasil uji aktivitas protease menunjukan terdapat penurunan aktivitas enzim tripsin setelah diberi protein rekombinan SPINK2 dengan konsentrasi optimum 0,6 mM.
Kesimpulan: Protein rekombinan SPINK2 telah berhasil dikonstruksi secara sintetis, diklon pada vektor plasmid pQE80L, diekspresikan pada E.coli strain BL21 kompeten, dan diketahui dapat menghambat aktivitas enzim protease dengan konsentrasi 0,7 mM.
.....Background: The process of spermatozoa maturation requires interaction between proteins synthesized and secreted by the epididymal epithelium to the lumen in a particular area. The interaction between these protein produces a microenvironment for maturation process of spermatozoa. In this condition, it takes genes that are specifically expressed. Spink2 in one of the genes known have a role in the maturation of spermatozoa and expressed in the epididymis. The SPINK2 role study requires sufficient protein for characterization. The aim of this study was to clone, express, and protease assay of the recombinant protein SPINK2.
Methods: In this study the mSpink2 gene was constructed synthetically. The plasmid pQE80L was used as an expression vector and the cell Escherichia coli strains used were Top10 and BL21. The cloning process begins with transformation of the plasmid pQE80L-mSpink2 to a competent E. coli Top10. The expression process was carried out by adding the induction agent Isopropyl-1-Thio-d-Galactopyranoside (IPTG),

purified by Ni-NTA chromatography. Then the protease activity assay was carried out with a wavelength 660 nm.
Results: The plasmid pQE80L-mSpink2 was confirmed to be cloned by restriction enzyme and sequencing analysis. The result showed that is formation of DNA with a length of 4709 bp and 258 bp. The SDS-PAGE and western blot result showed that SPINK2 was successfully expressed at the optimum time is 4th hour. There was a thick band with a size of 14 kDa. The SPINK2 recombinant protein was successfully purified and shown form the western blot. The result of the protease activity assay showed that there was a decrease in trypsin enzyme activity after being given SPINK2 recombinant protein with an optimum concentration is 0,6 mM.
Conclusion: Recombinant protein of SPINK2 has been successfully constructed synthetically, cloned, expressed, and tested for its protease inhibitor activity