UNIVERSITI PUTRA MALAYSIA

CLONING, EXPRESSION AND CHARACTERIZATION OF HEPATITIS B LARGE SURFACE ANTIGEN IN YEAST SYSTEM

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BY

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Chairman: **Tan Wen Siang, Ph. D.**

Faculty: **Biotechnology and Biomolecular Sciences**

Hepatitis B virus (HBV) infection is a worldwide health problem, which can lead to severe liver disease mainly hepatocellular carcinoma and cirrhosis. Hepatitis B surface antigen (HBsAg) is a glycoprotein found on the surface of the virus and its presence in the blood is an indicator of positive hepatitis B infection. The existing commercially available vaccine is based on the produced small HBsAg (S-HBsAg) via recombinant DNA technology. Although it is effective, about 5-10% of normal individuals demonstrates no or low response to the standard vaccination schedule. The efficacy of the large HBsAg (L-HBsAg) derived vaccine may differ from S-HBsAg, but such study needs to be conducted to evaluate the potential of L-HBsAg. To date, there is no

information on the expression of L-HBsAg intracellularly. Therefore, this study was carried out to express the L-HBsAg protein in yeast *Pichia pastoris* (*P. pastoris*) intracellularly. The *L-HBsAg* gene is inserted into the pPICZ C plasmid and introduced into *P. pastoris*. The positive clones were confirmed by DNA sequencing. Small scale expression of the recombinant L-HBsAg was analyzed by using Western blotting. The glycosylated L-HBsAg of about 42 kDa was detected by Western blotting and its concentration increase from 24 hours to 72 hours sample. The production of L-HBsAg was then scaled up and purified by using sucrose density gradient centrifugation. ELISA showed that the purified L-HBsAg was antigenic. Electron microscopic analysis revealed that the L-HBsAg assembled into spherical particles about 25 nm. The L-HBsAg produced in *P. pastoris* provides an alternative to the vaccines available in market.

PENGKLONAN, PENGEKSPERESAN DAN PENCIRIAN ANTIGEN PERMUKAAN L VIRUS HEPATITIS B DI DALAM YIS

Oleh

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Infeksi virus hepatitis B (HBV) merupakan masalah kesihatan sedunia, di mana ianya boleh mendatangkan penyakit-penyakit yang lebih serius seperti barah hati dan sirosis. Antigen permukaan virus hepatitis B (HBsAg) adalah glikoprotein yang terdapat di permukaan virus dan kehadirannya di dalam darah menandakan individu yang positif terhadap infeksi HBV. Vaksin komersil yang sedia ada dihasilkan daripada antigen permukaan S (S-HBsAg) menggunakan teknologi rekombinan DNA. Walaupun penggunanaan vaksin ini sangat efektif, masih terdapat kira-kira 5-10% daripada individu normal menunjukkan tiada atau tahap tindakbalas yang rendah terhadap vaksin tersebut. Keberkesanan vaksin yang dihasilkan daripada antigen permukaan L (L-HBsAg) berkemungkinan berbeza dengan S-HBsAg. Oleh itu, kajian terhadap potensi

L-HBsAg perlu dijalankan. Sehingga kini, tiada maklumat mengenai pengeksperesan L-HBsAg di dalam *Pichia pastoris* (*P. pastoris*) secara intrasellular. Oleh itu, kajian ini dijalankan untuk mengekspresi L-HBsAg dalam *P. pastoris* secara intrasellular. Gen *L-HBsAg* diselitkan ke dalam plasmid pPICZ C dan dimasukkan ke dalam *P. pastoris*. Klon positif dikenalpasti dengan teknik jujukan DNA. Pengeksperesan rekombinan L-HBsAg pada skala kecil dianalisis menggunakan kaedah pemblotan Western. Keputusan menunjukkan kehadiran protein bersaiz kira-kira 42 kDa (glikosilasi) dengan kepekatan meningkat daripada sampel 24 ke 72 jam. Kajian diteruskan dengan pengeskperesan L-HBsAg pada skala besar dan penulenan protein dengan menggunakan pengemparan gradiasi ketumpatan sukrosa. Ujian ELISA menunjukkan L-HBsAg adalah antigenic. Analisis menggunakan mikroskop elektron menunjukkan L-HBsAg menghasilkan zarah berbentuk sfera yang bersaiz kira-kira 25 nm. Dengan yang demikian, L-HBsAg yang dihasilkan di dalam *P. pastoris* merupakan alternatif terhadap vaksin yang berada di pasaran.

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I certify that a Thesis Examination Committee has met on (13 August 2010) to conduct the final examination of Fazia Binti Mohamad Sinang on her thesis entitled "Cloning, Expression and Characterization of Hepatitis B Large Surface Antigen in Yeast System" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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FAZIA BINTI MOHAMAD SINANG

DATE: 13 AUGUST 2010

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