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WARAPORN JAIPORKHUAN : HETEROLOGOUS EXPRESSION OF THE GENE ENCODING HEPATITIS B VIRUS SURFACE ANTIGEN IN YEAST. THESIS SUPERVISORY COMMITEE : CHUENCHIT BOONCHIRD, Ph.D., KAVI RATANABANANGKOON, Ph.D., SOAVANEE DHARMSTHITI, Ph.D. 131 p. ISBN 974-589-184-3

The PreS2+S gene of hepatitis B virus was inserted into yeast plasmid vector YEp52-1 which was constructed in this study and pYES2 which is a commercial plasmid. They contain galactose inducible *GAL10* and *GAL1* promoter, *CYC1* terminator (both plasmids), and auxotrophic selectable marker *LEU2* and *URA3*, respectively. *Saccharomyces cerevisiae* strain JEL-1 and BJ5462 were used as hosts for gene expression. Both strains of *S. cerevisiae* are vacuolar protease-deficient strains with *prb1* and *pep4* mutation. The expression of the PreS2+S gene product was measured by ELISA. The amounts of HBsAg expressed by plasmid YEp52-1 in JEL-1 and BJ5462 hosts were 14.55-21.00 ng/ml and 0.85-0.91 ng/ml, respectively. Plasmid pYES2 did not express HBsAg in both hosts. Western blot analysis of the PreS2+S gene product in host JEL-1 was shown to have molecular sizes of 34 kDa and 37 kDa when detected with the three kinds of antibodies, i.e., anti-S polyclonal antibody, anti-S and anti-PreS2 monoclonal antibody. After endoglycosidase H digestion, the sizes were reduced to 31 kDa and 34 kDa suggesting that the products were glycosylated and contained the N-linked glycan. Furthermore, the intact PreS2+S gene product was detected after extraction in the absence of protease inhibitor and prolong incubation at least for 1 h at 4°C indicating that the protein was not degraded by yeast protease. The pHSA activity of PreS2 protein was also demonstrated. The maximum HBsAg production of recombinant yeast of JEL-1 host was 22.91 μg/L at late log phase.