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SUNSANEE FOO-TRAKOON; STUDY ON THE EXPRESSION OF HEPATITIS B SURFACE AND ITS MODIFIED GENES IN *SACCHAROMYCES CEREVISIAE*. THESIS ADVISORS: CHUENCHIT BOONCHIRD. Ph.D., WATANALAI PANBANGRED, Ph.D., SAKORN MONGKOLSUK, Ph.D. 170 p ISBN 974-664-526-9.

The middle hepatitis B surface antigen (M HBsAg) comprises two parts, Pre-S2 and S regions, encoded by the Pre-S2+S gene. The S region contains the antigenic determinant *a* whereas the Pre-S2 region contains the epitopes for virus-neutralization and immunization and it can enhance immunogenicity of the S region. However, the Pre-S2 harbors protease sensitive site (Arg_{48}-Thr_{49}) which could result in proteolytic cleavage of the M HBsAg when the antigen is produced in yeast. In this work, the protease sensitive site in the Pre-S2 region was removed by *in vitro* mutagenesis to obtain Pre-S2d+S gene. The Pre-S2d+S gene was transformed into *S. cerevisiae* JEL-1 strain, expressed by *GAL*10p and the yeast protein extract, obtained from mid-logarithmic phase culture, was characterized by Western blot analysis using mouse anti-Pre-S2 monoclonal antibody, polymerized human albumin (pHSA) binding activity and determination of the S HBsAg by ELISA assay. It was found that the modified M HBsAg showed the same characteristic as the wild type M HBsAg. Study on the stability of M HBsAg to yeast proteolysis by incubation of yeast protein extract at 4 °C in the absence of protease inhibitor for period of time suggested that the modified M HBsAg was more stable. The expression of the Pre-S2+S gene by constitutive (*TP*Ip and *MF*α1p) and inducible (*GAL*10p) promoters were compared in the JEL-1 strain. It was found that the *GAL*10p gave HBsAg production higher than the *TP*Ip and *MF*α1p about 174 and 14 times, respectively. Moreover, study on cultivation and induction conditions for *GAL*10p expression demonstrated that 0.1% glucose would be enough to support initial growth phase and 0.25% galactose would be the minimal concentration for induction phase. In small batch cultivation, the maximum production of HBsAg in these conditions was about 79 ng/mg protein (16 μg/l culture) at 20 h of cultivation. In addition, the Pre-S2+S gene expression by *MF*α 1p was investigated in the SH2501 strain, *MAT***a**, *hml*α2–102, *HMR***a**, *sir*3-8^{ts}, mutant, of which *MF*α1p could be controlled with dual mode by mating type switching and temperature shift. The maximum production of HBsAg by *MF*α1p in this strain was about 80 and 91 ng/mg protein (16 and 18 μg/l culture) when grown with shift (25 °C to 30 °C) and constant (30 °C) temperature at 21 and 18 h of cultivation, respectively.

Therefore, the modified PreS2+S gene from this study would have potential for production of stable M HBsAg. However, the expression level by *GAL*10p and *MF*α 1p was still low.