STUDY ON THE EXPRESSION OF THE MIDDLE HEPATITIS B SURFACE GENE BY TRUNCATED \textit{HXT7} PROMOTER IN \textit{SACCHAROMYCES CEREVISIAE}. \\

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ABSTRACT \\

The \textit{HXT7} gene in \textit{Saccharomyces cerevisiae} encodes the high-affinity glucose transporter. This gene is derepressed in low concentration glucose medium or non-fermentable carbon source mediums such as glycerol and ethanol. However, when the 5' region of the \textit{HXT7} promoter is deleted, the truncated promoter, behaves like the constitutive promoter even in high concentration of glucose medium. The objective of this work is to investigate the expression of a heterologous gene by the truncated \textit{HXT7} promoter using the gene encoding middle hepatitis B surface antigen as a model gene. The \textit{PreS2d+S}, was inserted into plasmid pFL44-1 containing truncated \textit{HXT7} promoter, \textit{PGK1} terminator and \textit{URA3} selectable marker. Two recombinant plasmids were constructed: one was pEB-H2 harboring \textit{PreS2d+S} in which the native 5' upstream sequence from HBV origin was included and another one was pEB-H3 harboring the m \textit{PreS2d+S} in which the native 5' upstream sequence was replaced by yeast Kozak consensus. The expression of the \textit{PreS2d+S} gene was studied in \textit{S. cerevisiae} strain BJ5462 and CEN.PK113-6B. The recombinant yeasts were cultivated in a synthetic medium with 2% glucose as carbon source, and the M HBsAg was analyzed by Western Blot analysis using PreS2 mAb and quantified by ELISA method. The recombinant yeast BJ5462 could produce M HBsAg with correct size or about 34 and 37 kDa whereas the recombinant yeast CEN.PK113-6B produced M HBsAg as degraded form. The comparison of the M HBsAg production by the recombinant yeast BJ5462 harboring \textit{PreS2d+S} gene with different 5' upstream sequence revealed that the gene with yeast Kozak consensus sequence produced M HBsAg 7-fold higher than the gene with native 5' upstream sequence. Time-course of growth and M HBsAg production by the recombinant yeast in the medium with various carbon sources demonstrated that the M HBsAg was produced in parallel to logarithmic phase in glucose medium and the production reached maximal level in late logarithmic phase. In addition, the M HBsAg level was increased in diauxic growth phase when glucose concentration was limited. Furthermore, M HBsAg level was increased when the cells were cultivated in 2% glucose transferred to 2% ethanol as compared to 2% ethanol alone. The increase of M HBsAg level was highest when 2% ethanol was added in late logarithmic phase. Self-made synthetic medium with 2% glucose transferred to 2% ethanol could improve the maximal level of M HBsAg production to 408.92±16.50 µg/l which was about 2.5-fold higher than the medium with 2% glucose alone. The M HBsAg protein expressed by the truncated \textit{HXT7} promoter in the recombinant yeast BJ5462 was intact throughout the period of cultivation. \\

In conclusion, this study demonstrated that the expression of hepatitis B surface gene by truncated \textit{HXT7} promoter could be achieved in yeast strain BJ5462 when cultivated in a medium with glucose combined with ethanol. The production of M HBsAg was growth associated and maximal level was obtained at late logarithmic phase. Modification of 5' upstream sequence and cultivation in medium with glucose combination with ethanol improved the production level. \\

KEY WORDS : \textit{S. CEREVISIAE} / HETERLOGOUS PROTEIN / HBsAg / TRUNCATED \textit{HXT7} PROMOTER \\

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