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Cloning, Intracellular Expression, and Characterization of Recombinant mHBsAg from Hepatitis B Virus Isolate Indonesia in *Pichia pastoris*

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Abstract

Hepatitis B is a serious infection disease caused by the hepatitis B virus (HBV). Vaccination is an effective way to prevent hepatitis B infection. The first marker that appears of HBV infection is HBsAg (Hepatitis B surface Antigen). HBsAg is composed of sHBsAg (S), mHBsAg (pre-S2 and S), and lHBsAg (preS1, preS2, and S). Indonesia still imports the sHBsAg protein to meet the national needs. Therefore, the original HBsAg Indonesian isolate with genotype B3 subtype *adw2* need to be produced by recombinant protein production systems. This research is first step to develop new generation of hepatitis B vaccine, focus on the cloning, intercellular expression, and characterization of mHBsAg using *Pichia pastoris* cells. Synthetic DNA encoding mHBsAg was purified from pJ902_VM plasmid, ligated into pAO815, and produce pAOMHBs1. One cassette expression on pAOMHBs1 was transformed to *Pichia pastoris* cells to produce clones *Pichia pastoris* with mHBsAg integration, its expressed intracellularly by methanol as inducer. In this study, characterization one cassette expression pAOMHBs1 was done by PCR colony, restriction, and sequencing analysis showed that 100% homolog characteristics with initial design. mHBsAg protein was expressed, verified by cell lysates were characterized using Southern Blot, SDS-PAGE, and Western Blot analysis.

Keywords : M protein, Indonesia's isolat, *Pichia pastoris*, Vaccine, Hepatitis B.

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