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Optimization of Hepatitis B Surface Antigen Purifications to Improving the Production of Hepatitis B Vaccines on Pichia pastoris

Authors : Rizky Kusuma Cahyani

Abstract : Hepatitis B is a liver inflammatory disease caused by hepatitis B virus (HBV). This infection can be prevented by vaccination which contains HBV surface protein (sHBsAg). However, vaccine supply is limited. Several attempts have been conducted to produce local sHBsAg. However, the purity degree and protein yield are still inadequate. Therefore optimization of HBsAg purification steps is required to obtain high yield with better purification fold. In this study, optimization of purification was done in 2 steps, precipitation using variation of NaCl concentration (0,3 M; 0,5 M; 0,7 M) and PEG (3%, 5%, 7%); ion exchange chromatography (IEC) using NaCl 300-500 mM elution buffer concentration.To determine HBsAg protein, bicinchoninic acid assay (BCA) and enzyme-linked immunosorbent assay (ELISA) was used in this study. Visualization of HBsAg protein was done by SDS-PAGE analysis. Based on quantitative analysis, optimal condition at precipitation step was given 0,3 M NaCl and PEG 3%, while in ion exchange chromatography step, the optimum condition when protein eluted with NaCl 500 mM. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis indicates that the presence of protein HBsAg with a molecular weight of 25 kDa (monomer) and 50 kDa (dimer). The optimum condition for purification of sHBsAg produced in Pichia pastoris gave a yield of 47% and purification fold 17x so that it would increase the production of hepatitis B vaccine to be more optimal.

Keywords : hepatitis B virus, HBsAg, hepatitis B surface antigen, Pichia pastoris, purification
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