

## CHAPTER 6

### CONCLUSION

In this experiment, haemagglutinin (H5N1 HA1\_OPT or H5N1 HA2) gene was cloned and expressed in recombinant *P. pastoris*. Haemagglutinin protein was an antigenic proteins produced by recombinant protein which used for diagnosis and epidemiologic study of avian influenza (AI). All of results were concluded as described below.

1. The haemagglutinin (H5N1 HA1\_OPT or H5N1 HA2) gene was successfully cloned into pPICZA or pPICZαB expression vector and transformed in to *P. pastoris*. The transformation efficiency of transformants of *P. pastoris* strain GS115 containing recombinant vector (pPICZA-H5N1 HA2) was  $1.11 \times 10^3$  cells/μg DNA and the transformation efficiency of transformants of *P. pastoris* strain X33 containing recombinant vector (pPICZA-H5N1 HA1\_OPT, pPICZαB-H5N1 HA1\_OPT) was  $2.02 \times 10^3$  cells/μg DNA and  $5.19 \times 10^3$  cells/μg DNA, respectively. While the transformation efficiency of *P. pastoris* containing recombinant vector (pPICZαB-H5N1 HA2) could not calculated.

2. Only the recombinant haemagglutinin (pPICZA-H5N1 HA2) was successfully expressed by cultivated *P. pastoris* strain GS115 containing H5N1 HA2 gene in BMGY and induced with methanol to final concentration of 1% (v/v) every 12 hours for 3 days. The cells were collected to extraction of endogenous protein from yeast cells by ultrasonicator and the cell lysate was used to purify of H5N1 HA2 protein using HisPur™ Ni-NTA Spin Column under native condition.

3. Western bolt analysis which test by HisDetector™ Western Blot Kits, Anti-myc antibody and PO3 (positive serum) revealed that the H5N1 HA2 protein presented with

immunostained bands with molecular weight approximately 35-40 kDa. The purified protein was not reacted with negative serum in the western bolt analysis

4. Finally, the *P. pastoris* strain GS115 containing H5N1 HA2 gene which used in this experiment could be express haemagglutinin domain of avian influenza A virus which used for antigen production used in epidermologic study.



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