Thesis Title Selection of *Staphylococcus aureus* isolates and production of protein A for clinical application.

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ABSTRACT

Since staphylococcal protein A is capable to react with Fc portion of several mammalian IgG, it is now widely used as an useful tool for immunosorbent and other purposes in various fields of biological science. As the rapid increasing of newly developed test, demand of protein A becomes endlessly eminent. This study was designed to construct a laboratory scale for protein A production from a clinical isolates of staphylococci to supply the locally applicable use.

Secreted and cell-bound protein A were screened among clinical staphylococci by plating technique on heart infusion agar contained 2% human serum and co-hemagglutination using sensitized sheep erythrocytes, respectively. Production of protein A was performed via specialized cultivation where a cellophane bag filling with brain heart infusion broth was enclosed. Protein A was purified by one step IgG affinity chromatography.

By screening 281 clinical isolates, it was revealed that 93% of coagulase positive staphylococci and 76% of methicillin resistant *Staphylococcus aureus* (MRSA) produced protein A. Among these,

86.1% and 59.6% of coagulase positive staphylococci demonstrated cell-bound and extracellular secreted protein A, respectively. While 48.1% and 72.2% of MRSA bore the same repetitive form of protein A, respectively. Staphylococcal protein A was found actively secreted throughout the exponential growth. A clinically derived *S. aureus* (S- 123), produced 170.5 nanogram of protein A per 10^8 CFU, was selected and subjected for protein A production. An amount of 35 mg of purified protein A could conveniently be generated via 1 litre of specialized culture. One step affinity chromatography evoked 70-90% protein A yield with the purification folds of 137-149.

Apparently molecular weight of purified protein A was 41-42 KDa on gel filtration. Upon brief boiling, lower molecular mass with fully functional protein A of 38 and 36 KDa were obtained on SDS-PAGE. This home-made protein A was further chemically conjugated to horseradish peroxidase and subsequently used as reagent for ELISA to detect IgG and differentiate melioidosis. The results showed that home-made protein A peroxidase conjugate could detect human IgG down to 28 nanogram. It also generated compatible result as compared to commercial rabbit anti-human immunoglobullin horseradish peroxidase conjugate to differentiate melioidosis from healthy individual. Clearer background was demonstrated by home-made protein A peroxidase conjugate. Coupling of home-made protein A to Sepharose 4B was as well performed and used for affinity IgG purification. The single affinity step yield 83% of purified IgG while the multiple step conventional method using ion exchanger, Sepharose CL6B evoked only 15.6% yield.

Home-made protein A was fully economical as the cost effectiveness estimation. Without including the labour work, one milligram cost only 22.30 Baht while the commercial one is 27 times more expensive (600 Baht/mg).