Thesis title: Evaluatioin of a partially purified cholesterol esterase for clinical assay of cholesterol

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ABSTRACT

Serum cholesterol determination is a routine diagnostic method for arteriosclerosis. The enzymatic determination of cholesterol requires three enzymes, namely, cholesterol esterase, cholesterol oxidase and peroxidase. The objective of this project was to investigate a new source of cholesterol esterase suitable for diagnostic application.

Forty five isolates of bacteria and sixteen isolates of moulds were screened for extracellular cholesterol esterase. The isolate that produced the highest yield of the enzyme was selected and was identified as *Pseudomonas* sp. based on Bergey's manual. The enzyme was partially purified by 2 methods: one method by the combination of DEAE-cellulose column and Sephadex G-200 gel column chromatography, and the other method by calcium chloride precipitation. The purification fold of the enzyme were about 24 and 13 respectively. The specific activity of the partially purified

enzyme was about 1.2 unit/mg protein with 75% recovery for both methods of purification.

The partially purified enzyme was studied for its physical and biochemical properties, and the results were the followings: molecular weight was about 600,000 daltons by gel filtration, pH optimum 7.0-9.0, temperature optimum 50°C, pH stability in the range 5.0-10.0, K_m value 3.3×10^{-5} M, stable at 4°C and in frozen condition for at least 2 months, the enzyme was able to hydrolyze serum cholesterol esters to the same extent as alkaline hydrolysis.

These results suggest that cholesterol esterase from this pseudomonad was potentially suitable for further development in diagnostic application.