Thesis Title Evaluation the Performance Characteristic of Cholesterol Oxidase Isolated from *Streptomyces sp.*, *Pseudomonas sp.*, *Brevibacterium sp.* and *Cellulomonas sp.* for the Kinetic Determination of Total Serum Cholesterol

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ABSTRACT

The enzymatic method for serum cholesterol determination can be either the endpoint or the kinetic method. Lolekha and Juntaveeserirat suggested that *Streptomyces sp.* cholesterol oxidase was a superior source for the enzymatic endpoint method than the enzyme obtained from *Nocardia sp.* and *Pseudomonas sp.* because of its economical cost and the longest stability.

The objective of this study was to find the suitable sources of cholesterol oxidase for serum cholesterol assay by the kinetic method. The Michaelis constant (K_m) of *Streptomyces sp.*, *Pseudomonas fluorescens*, *Brevibacterium sp.* and *Cellulomonas sp* cholesterol oxidase were determined and compared. The *Brevibacterium* cholesterol oxidase gave the highest K_m value (230.3×10^{-4} M), followed by the K_m values of *Streptomyces sp.* (2.17×10^{-4} M), *Cellulomonas sp.* (0.84×10^{-4} M) and *Pseudomonas sp.* (0.61×10^{-4} M).

mmol/L (1000mg/dL). The K_m values of *Streptomyces sp.*, *Pseudomonas sp.*, and *Cellulomonas sp* cholesterol oxidase were too low to achieve the first order kinetics over a wide range of serum cholesterol. Cholesterol linearity obtain from *Streptomyces sp* (1.56 mmol/L or 60 mg/dL), *Pseudomonas sp.* (1.04 mmol/L or 40 mg/dL) *and Cellulomonas sp* (1.04 mmol/L) cholesterol reagents was too low; they were not suitable for detection of cholesterol concentration normally present in human serum (upto 5.2 mmol/L or 200 mg/dL). To increase the K_m value of cholesterol oxidase, the inhibitor effect of dichlorophenol isomers was examined. Only 3,4 dichlorophenol could be a competitive inhibitor for *Streptomyces sp.* cholesterol oxidase; its K_m value was raised from 2.17×10^{-4} M to 24.89×10^{-4} M. The *Streptomyces* cholesterol reagent with the addition of 3,4 dichlorophenol, 5 mmol/L raised cholesterol linearity up to 20.72 mmol/L (800 mg/dL).

In conclusion, *Brevibacterium sp.* and *Streptomyces sp.* (with the addition of 3,4 dichlorophenol) cholesterol oxidase are suitable sources for using in cholesterol reagent in the detection of human serum cholesterol by the kinetic method. The reagent containing *Brevibacterium sp* cholesterol oxidase has lower cost (2.05 bath/ mL) and higher linearity (25.9 mmol/L) compared to *Streptomyces sp* cholesterol oxidase (32.80 bath/mL, 20.72 mmol/L). However, the disadvantage of using *Brevibacterium sp.* enzymatic reagent is due to its use of high sample volume (30 µL) compared to *Streptomyces sp* (3µL).