

**THE EFFECT OF TEMPERATURE AND INDUCER CONCENTRATION ON  
THE EXPRESSION OF RECOMBINANT PHOSPHOLIPASE A2 IN  
BACTERIAL EXPRESSION SYSTEM**

**BY :**

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## ABSTRACT

### THE EFFECTS OF TEMPERATURE AND INDUCER CONCENTRATIONS ON THE EXPRESSION OF RECOMBINANT PHOSPHOLIPASE A<sub>2</sub> IN *Escherichia coli*

Phospholipase A<sub>2</sub> is an enzyme that catalyzed the hydrolysis of long chain triglycerides producing lysophospholipids and free fatty acids. Its catalytic properties which act as powerful emulsifier causes it to be widely used in various industrial application including foods, cosmetics as well as pharmaceuticals industry. Unfortunately, most of the enzyme currently used by the manufacturer is derived from porcine which is forbidden to be consumed by Muslims. Therefore this project was aimed to produce phospholipase A<sub>2</sub> enzyme in *E. coli* using recombinant technology as well as to determine the optimum incubation temperature and to examine expression of PLA<sub>2</sub> in *E. coli* at different inducer concentrations for commercialization. This is to produce PLA<sub>2</sub> enzyme in *E. coli* which fulfilled the *Halal* concept in Islamic religion. There were five clone of *E. coli* used in this study. Three of the clones harboring *Pla*<sub>2</sub> gene. Meanwhile, another two clones act as negative and positive control respectively. 100 µL overnight cultured was transferred into new bottle containing LB broth. After two hours, the cultures were supplemented with arabinose at two different concentrations which were 0.02% and 0.2% to induce the expression of the desired protein. Samples that have been collected were run through SDS-PAGE electrophoresis. Concurrently positive and negative control strains which do not contain plasmid and plasmid without *Pla*<sub>2</sub> gene were also run. Results gained indicates that only induced samples showed expression of PLA<sub>2</sub> protein which were pBAD Thio – TOPO *Pla*<sub>2</sub> (-TAG 5) and pBAD Thio – TOPO *Pla*<sub>2</sub> (-TAG 8) either in 0.02% or 0.2% concentrations of arabinose. pBAD Thio-TOPO *Pla*<sub>2</sub> (-TAG 16) is believed to be mutated. It also shows positive correlation between the growth rate and the amount of protein produced. However, significant effect towards the amount of protein being expressed was also found to be given by the incubation temperature. Result shows that incubation temperature of 37°C produced higher protein amount when compared to incubation temperature of 27°C.

*Keywords:* Phospholipase A<sub>2</sub>; *Escherichia coli*; Inducer; Temperature