UNIVERSITI TEKNOLOGI MARA

THE KINETIC GROWTH STUDY OF *Escherichia coli* IN PRODUCTION RECOMBINANT PLA_2

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ABSTRACT

Recombinant *E. coli* is seen here as an alternative toward production of phospholipase A_2 enzymes. The recombinant PLA_2 of pBADTOPO-pla_2 5 and pBADTOPO-pla_2 8 were successfully cloned and sequence in previous study. The clones were recognized using SDS-PAGE and the result found that the recombinant PLA_2 was successfully expressed as a soluble protein by arabinose induction. Study on kinetics growth of recombinant PLA_2 creates more understanding on their biological activity in which can be used for future optimization in fermentation process. The kinetics growth of recombinant PLA_2 was study in shake flask culture and their growth pattern was observed through cells viability, Bradford protein assays and dry cell weight. The culture was grown using suitable medium with suitable growth condition. The catalytic activity of recombinant pBADTOPO-pla_2 5 and pBADTOPO-pla_2 8 was then tested through $sPLA_2$ assay and found to display enzymatic activity. Results from kinetics study reveal that the recombinant proteins shown normal growth pattern with high protein production at log phase.

CHAPTER 1

INTRODUCTION

There are many host system available in production of recombinant protein including bacteria, yeast, insect cell, plant and others. The most popular host for producing recombinant protein is the bacterium *Escherichia coli*. The transformation of *E. coli* with foreign DNA is uncomplicated with well-established genetic manipulation methods. In addition, *E. coli* are preferred mainly due to their simplicity, safety, and known genetic properties, thus generation of stable cell lines is a speedy process. The major advantage of *E. coli* is the ability to produce proteins in large quantities and it can grow very quickly compared to mammalian cells. (Leonhartsberger, 2006). Demain & Vaishnav, (2009) stated that the least expensive, easiest and quickest expression of protein can be carried out in *E. coli*. With the advent of genetic engineering, recombinant proteins entered the market, which totally changed the situation of the industry. Through the use of recombinant DNA, important genes especially mammalian genes, could be amplified and cloned in foreign organisms.