ABSTRACT

Danti Nur Savitri. 24020122140193. Optimization of Recombinant Lactate Dehydrogenase Production in *Escherichia coli* BL21 (DE3) through Variations in Temperature, Inducer Concentration, and Post-Induction Time. Under the Supervision of Anto Budiharjo and Radityo Pangestu.

Lactate dehydrogenase (LDH) is a crucial enzyme in lactic acid biosynthesis, making a production strategy essential for obtaining high catalytically active enzyme. This study aimed to investigate the effects of temperature, IPTG concentration, and post induction time on the production of recombinant D-LDH and L-LDH from *Pediococcus pentosaceus* InaCC B159, to identify the optimal production conditions, evaluate the enzymatic activity, and examine the effect of *ldhD* and *ldhL* genes insertion on lactic acid production in *Escherichia coli* BL21(DE3). The *ldhD* and *ldhL* genes were expressed in *E. coli* BL21 (DE3) using the pET-28a(+) vector. Optimization was performed by varying the temperature (25, 30, and 37 °C), IPTG concentration (0,5, 1, and 2 mM), and post induction time (12, 18, and 24 h), with uninduced and empty vector controls at each optimization stage. Every stage consisted of five groups with three biological replicates in a completely randomized design (CRD). Lactic acid were determined in *E. coli* BL21 (DE3)-pET-28a-*ldhD*, *E. coli* BL21 (DE3)-pET-28a-*ldhL*, and *E. coli* BL21 (DE3)-pET-28a(+) cultures at 0, 9, and 48 h, each with triplicate. Protein expression was analyzed by SDS-PAGE and the Bicinchoninic Acid (BCA) assay, while enzyme activity was assessed from pyruvate to lactate conversion. The results showed that temperature, IPTG concentration, and post induction time significantly influenced protein production. The optimal production conditions for D-LDH were 25 °C, 0,5 mM IPTG, and 18 h post induction, while for L-LDH were 25 °C, 1 mM IPTG, and 18 h post induction. Under these conditions, D-LDH achieved an enzymatic activity of 5,88 U/mL with a specific activity of 0,30 U/mg, while L-LDH showed an enzymatic activity of 8,62 U/mL with a specific activity of 0,36 U/mg. Insertion of the *ldhD* and *ldhL* genes increased lactic acid production with D-lactate and L-lactate reaching 1,041 g/L and 0,636 g/L.

Keywords: *Pediococcus pentosaceus; lactate dehydrogenase; recombinant protein; lactic acid*