

## **Optimization of culture conditions for Mpt64 synthetic gene expression in Escherichia coli BL21 (DE3) using surface response methodology**

### **ABSTRACT**

MPT64 is a specific protein that is secreted by Mycobacterium tuberculosis complex (MTBC). The objective of this study was to obtain optimum culture conditions for MPT64 synthetic gene expression in Escherichia coli BL21 (DE3) by response surface methodology (RSM). The RSM was undertaken to optimize the culture conditions under different cultivation conditions (medium concentration, induction time and inducer concentration), designed by the factorial Box-Bhenken using Minitab 17 statistical software. From the randomized combination, 15 treatments and three center point repetitions were obtained. Furthermore, expression methods were carried out in the flask scale fermentation in accordance with the predetermined design. Then, the MPT64 protein in the cytoplasm of E. coli cell was isolated and characterized using sodium dodecyl sulfate polyacrilamide electrophoresis (SDS-PAGE) then quantified using the ImageJ program. The optimum conditions were two-fold medium concentration (tryptone 20 mg/mL, yeast extract 10 mg/mL, and sodium chloride 20 mg/mL), 5 h of induction time and 4 mM rhamnose. The average concentration of recombinant MPT64 at optimum conditions was 0.0392 mg/mL, higher than the predicted concentration of 0.0311 mg/mL. In conclusion, the relationship between the selected optimization parameters strongly influenced the level of MPT64 gene expression in E. coli BL21 (DE3).

**Keyword:** Environmental science; Microbiology; MPT64; Response surface methodology; Box-Behnken design; Rhamnose; Medium; Induction