Construction and expression of synthetic gene encoding MPT64 as extracellular protein in Escherichia coli BL21 (DE3) expression system

ABSTRACT

Aim: This study was purposed to produce MPT64 protein extracellularly by contructing pelB signal peptide into expression vector containing synthetic gene of MPT64 from Mycobacterium tuberculosis H37Rv Methods: The mpt64 synthetic gene was derived from gene sequence of M. tuberculosis H37Rv. Therefore, the mpt64 gene sequence need codon optimization using a graphical codon usage analyzer (GCUA) to improve gene expression in E. coli. The optimized codon then design and construct into an E. coli expression vector (pD861-SR) containing such component that high replication, strong RBS, induction system with rhamnose and supplemented with kanamycin selection markers. The pelB gene, a gene coding for extracellular proteins was inserted into the vector. The transformation of pD861-SR plasmid into E. coli BL21 (DE3) was conducted using electroporation method and the isolated plasmid was verified by sequencing method. The overproduction of MPT64 protein was induced by 4 mM rhamnose in different time of induction. Followed by characterization of MPT64 protein using sodium dodecyl sulfate polyacrilamide electrophoresis (SDS-PAGE). Results: The gene sequencing results showed there was no nucleotide changes in the sequences of mpt64 and pelB genes. Based on SDS PAGE analysis, the pelB signal peptide could translocate the MPT64 protein into the medium as an extracellular protein with a molecular weight of 24 kDa. Conclusion: The expression of mpt64 gene combined with pelB as signal peptide could be a choice design in plasmid recombinant construction to yield MPT64 as extracellular protein.

Keyword: MPT64; Mycobacterium tuberculosis H37Rv; pelB; Extracellular; Escherichia coli