EFFICIENT HETEROLOGOUS EXPRESSION AND ONE-STEP PURIFICATION OF FULLY ACTIVE C-TERMINAL HISTIDINE-TAGGED URIDINE MONOPHOSPHATE KINASE FROM *MYCOBACTERIUM TUBERCULOSIS*

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Abstract. Tuberculosis has long been recognized as one of the most significant public health problems. Finding novel antituberculous drugs is always a necessary approach for controlling the disease. *Mycobacterium tuberculosis pyrH* gene (Rv2883c) encodes for uridine monophosphate kinase (UMK), which is a key enzyme in the uridine nucleotide interconversion pathway. The enzyme is essential for *M. tuberculosis* to sustain growth and hence is a potential drug target. In this study, we have developed a rapid protocol for production and purification of *M. tuberculosis* UMK by cloning *pyrH* (Rv2883c) of *M. tuberculosis* H37Rv with the addition of 6-histidine residues to the C-terminus of the protein, and expressing in *E. coli* BL21-CodonPlus®(DE3)-RIPL using an auto-induction medium. The enzyme was efficiently purified by a single-step TALON® cobalt affinity chromatography with about 8 fold increase in specific activity, which was determined by a coupled assay with the pyruvate kinase and lactate dehydrogenase. The molecular mass of monomeric UMK was 28.2 kDa and that of the native enzyme was 217 kDa. The enzyme uses UMP as a substrate but not CMP and TMP and activity was enhanced by GTP. Measurements of enzyme kinetics revealed the k_{cat} value of 7.6 ± 0.4 U mg^{-1} or 0.127 ± 0.006 sec^{-1}.The protocol reported here can be used for expression of *M. tuberculosis* UMK in large quantity for formulating a high throughput target-based assay for screening anti-tuberculosis UMK compounds.

Keywords: *Mycobacterium tuberculosis*, uridine monophosphate kinase, antituberculous drugs, pyrimidine, protein purification

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