Cloning, Overexpression and Purification of UMP Kinase from *MycobacteriumTuberculosis*

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Millions of people have died from tuberculosis (TB), a chronic infectious disease mainly caused by *Mycobacterium tuberculosis*. Increasing numbers of co-infections with HIV, multidrug-resistant and extensively drug-resistant strains led to focus on the development of new drugs to combat this disease. Nucleotide biosynthesis is an essential step in the progression of TB, therefore UMP kinase, a protein of the pyrimidine metabolism, encoded by the *pyrH* gene, which catalyses the phosphorylation of UMP to UDP, is an attractive antitubercular drug target. The aims of this study were amplification and cloning of the *pyrH* gene, overexpression and purification of the recombinant enzyme, for further protein inhibitor development. The full-length *pyrH* coding region (786bp) was amplified by PCR and the fragment was cloned at the *NdeI* and *HindIII* restriction sites of the expression vector pET-23a+ (Novagen). The resulting plasmid was introduced into the *E. coli* BL21(DE3) strain and grown in TB medium at 30°C. The protein expression was obtained after 24h without induction of IPTG, and >90% of UMP kinase was observed in a soluble form. The recombinant UMP kinase was purified with anion exchange and hydrophobic interaction chromatography columns using a FPLC system. UMP kinase was obtained (40mg) with a purity higher than 95% as seen by Coomassie Blue staining on SDS/PAGE. These results will allow kinetic and structural studies of UMP kinase which represents an essential step in our efforts to characterize a target for anti-tuberculosis drug development.