The Enzyme Thymidylate Synthase (E.C. 2.1.1.45) of *Mycobacterium Tuberculosis:* Cloning and Expression in a Heterologous System.

Brancher, C.[1 2], ; Breda, A.[1]; Santos, D. S.[1]; Basso, L. A.[1]

1. Centro de Pesquisas em Biologia Molecular e Funcional .
Instituto Nacional de Ciência e Tecnologia em Tuberculose. PUCRS.
[2.] Faculdade de Farmácia.
Pontifícia Universidade Católica do Rio Grande do Sul, Rio Grande do Sul.

Tuberculosis (TB) remains a major global health concern. Its causative agent, Mycobacterium tuberculosis, has been estimated to infect approximately one-third of the world's population, and approximately 30 million people have died from the disease in the past decade. Thymidylate synthase (TS, EC 2.1.1.45) is a key enzyme for the de novo synthesis of DNA and as such a target for development of new medicines against TB. TS is a critical enzyme for DNA replication since it catalyses the de novo synthesis of thymidine monophosphate (TMP), a key nucleotide precursor for DNA synthesis.In this work, our specific goals were to amplify, clone and subsequently express the gene thyA that encodes M. tuberculosis TS protein.

We were able to amplify the thyA gene of Mycobacterium tuberculosis from its total genomic DNA, with the expected size of 792 bp. The amplified gene was correctly cloned into cloning and expression vectors, pCR-Blunt and pET-23a(+). The TS protein expression on the soluble fraction was observed with BL21(DE3) E. coli strain, TB medium, at 30ºC and 0.1mM IPTG induction. New tests will be performed to enhance expression conditions and cellular growth. These results will allow protein purification by high-performance liquid chromatography and biochemical assay for TS activity. Future work will also involve enzyme kinetics and thermodynamic studies for detailed characterization of the protein's biochemical proprieties. These will be important for characterizing the pyrimidine metabolism pathway in mycobacteria and for development of new drugs against TB.