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Cloning a Mycobacterial Acyltransferase Gene and Expressing the Protein in Escherichia coli

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Cloning a Mycobacterial Acyltransferase Gene and Expressing the Protein in *Escherichia coli*

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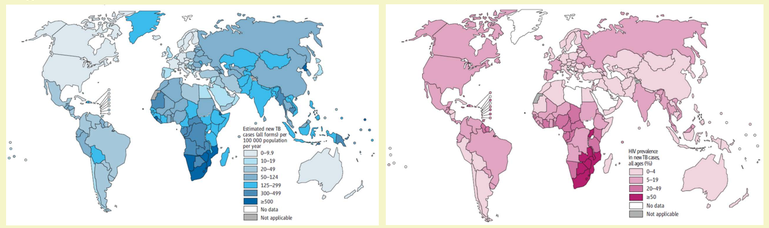
Abstract

Tuberculosis (TB) is a disease caused by bacillus *Mycobacterium tuberculosis* (*Mtb*) that destroys pulmonary tissue and ultimately causes death by suffocation [1]. Modern antibiotics such as rifampicin and isoniazid have proven futile against *Mtb* when it goes into its dormant state under hypoxic conditions in the granuloma because of its ability to survive on host lipids [2, 3]. *Mtb* is able to create a reservoir of triacylglycerol (TAG) by cleaving fatty acids from macrophage TAG, transporting them inside the phagosome, and subsequently reattaching the acyl groups to a glycerol backbone [2]. The genes involved in coordinating this process are thought to play a crucial role in the bacteria's survival. Although the gene associated with the final step of the acyltransferase pathway that attaches the third acyl group to the glycerol backbone has been studied, the genes involved in attachment of the first and second acyl groups have not been studied even though they are essential to the pathway. Therefore, we have cloned the acyltransferase gene that catalyzes the first step of the pathway and expressed it in *Escherichia coli* (*E. coli*) in order to understand how the protein functions. Expression of the mGPAT1 protein in *E. coli* has been confirmed and future studies will characterize the enzymatic activity of the protein.

Introduction

The World Health Organization (WHO) reports that about 2 billion people are latently infected by *Mycobacterium tuberculosis* (*Mtb*) with 8.6 million new diagnoses and 1.3 million deaths in 2012 with 0.3 million HIV related TB deaths (**Fig. 1**)**.** Among these new cases, an estimated 450,000 worldwide have been diagnosed with multiple drug-resistant tuberculosis (MDR-TB), against which first-line antibiotics have no effect, and 9.6% of those cases have extremely drug-resistant TB (XDR-TB), which are resistant to second-line antibiotics [3]. Typically TB infects alveolar macrophages in the lung and survives in a dormant state by sequestering lipids from the infected host cells, which become isolated from the rest of the pulmonary tissue inside a granuloma composed of lymphocytes, mononuclear phagocytes, and lipid rich macrophages all encapsulated in endothelial cells [2].

Figure 1: A. TB incidence rates in 2012 **B. HIV prevalence in new 2012 TB cases**



Many proteins play an integral part in the accumulation of TAG inside *Mtb* including those that esterify fatty acids to glycerol to form TAG. The protein responsible for attaching the third acyl group to the glycerol backbone has been studied and identified; however, the proteins involved in attaching the first and second acyl groups to the glycerol backbone have not been studied. Biochemical characterization of the enzyme involved in the attachment of the first acyl group to the glycerol backbone will be the particular aim of this research (**Fig. 2**)**.**

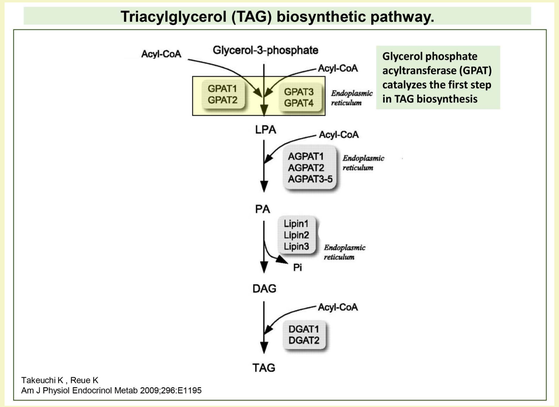


Figure 2: Biosynthetic pathway of triacylglycerol

Results

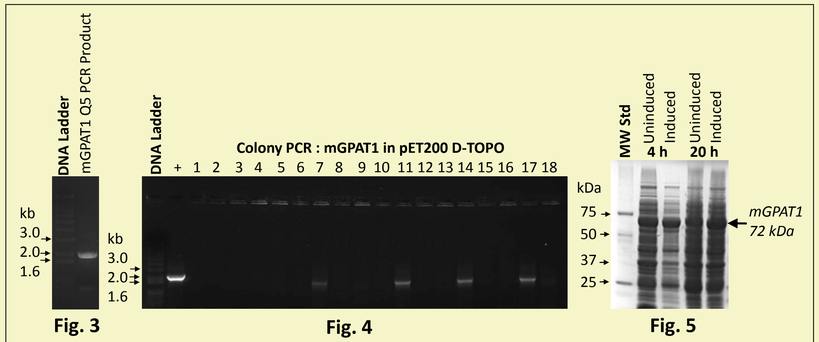


Figure 3: Polymerase chain reaction amplification and cloning of *mGPAT1* gene in pET200 D-TOPO expression plasmid vector.
Figure 4: Colony PCR analysis to detect insertion of *mGPAT1* gene in pET200 D-TOPO plasmid.
Figure 5: Expression of mGPAT1 protein in *E. coli* BL21 (Star) DE3 cells.

- mGPAT1 was amplified and cloned in pET200 D-TOPO expression plasmid vector (**Fig. 3**).
- A few colonies tested positive for the insert in the plasmid (**Fig. 4**).
- A band of expected size (72 kDa) for the mGPAT1 protein was present in *E. coli* cell lysates (**Fig. 5**).

Methods

mGPAT1 gene cloned by polymerase chain reaction (PCR) amplification using a high-fidelity polymerase Q5 (New England Biolabs, MA) from the genomic DNA of *Mtb* and inserted into the Champion pET200 D-TOPO expression vector (Life Technologies, NY).

E. coli TOP10 cells transformed with *mGPAT1*-pET200 construct screened by colony PCR to detect clones with the inserted *mGPAT1* gene in pET200 plasmid.

Agarose gel electrophoresis to detect colonies having plasmid with insert. DNA sequencing of plasmids with insert to rule out mutations in the cloned *mGPAT1* gene.

E. coli BL21 Star (DE3) cells transformed with *mGPAT1*-pET200 expression construct. Expression of mGPAT1 protein induced with Isopropyl-beta-D-thiogalactopyranoside (IPTG) for 4 h and 20 h at 37 °C. Uninduced cells did not receive any IPTG.

Aliquots of cell lysates were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to test if the protein was being expressed in the cell.

Discussion

The experimental results show that *mGPAT1* was successfully cloned into the expression plasmid pET200 D-TOPO. Analysis of cell lysates by denaturing protein gel electrophoresis confirmed that the mGPAT1 protein is indeed expressed in *E. coli*. Future experiments will focus on purifying the mGPAT1 protein and characterizing its enzymatic activity.

References

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3. *Global tuberculosis report 2013*. 2013, World Health Organization.

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