# Purification of Phenolic Glycolipid from *Mycobacterium tuberculosis*

Scott Vigil, Danny Hesser and Dr. Karen Dobos
Department of Microbiology, Immunology and Pathology
Colorado State University, Fort Collins, CO

Colorado State University

## Abstract

*Mycobacterium tuberculosis* (*Mtb*) is a very successful human pathogen, infecting one-third of the world's population. Although originally thought to have little to no genetic variation, *Mtb* has been recently shown to be distinguished to clades based on geographical distribution. Further, members of specific clades (and/or subclades) have been shown to possess differences in virulence. Several clades from Asia, Africa, and India were found to express phenolic glycolipid (PGL). PGL is absent in many North American and European clades due to gene deletion. The West Beijing strains of Mtb, HN878, W4, and W451, contain PGL as part of their cellular envelope. These strains demonstrated reduced production of important immune-mediating cytokines including tumor necrosis factor alpha and interleukin 12. Subsequent evidence demonstrated this was due to PGL. Thus, it is believed that PGL works in concert with other factors to enhance the virulence of *Mtb*. Purification of PGL from these strains will permit further studies on the molecular interactions of PGL during infection with *Mtb*. In our laboratory, we attempted to isolate PGL from *Mtb* strain HN878. Initially, a lipid extraction was performed with 1:2 chloroform: methanol. This extract was then analyzed via preparatory and analytical Thin-Layer Chromatography (TLC) plates with a 90:10 and 95:5 chloroform: methanol developing solution. These TLC plates were then sprayed with $CuSO_4$ and α-naphthol for sugar and carbon compound detection. Ultra-violet light was used to view and outline what is believed to be the major PGL band. This band was scraped off the TLC and will be extracted with diethyl ether to purify PGL. Once purified, we will perform studies with naïve and *Mtb*-infected macrophages to assess the role of PGL in the pro-inflammatory response during infection.

## Introduction

*Mycobacterium tuberculosis* causes the most widespread potentially lethal bacterial infection of humans.[2] *Mtb*, along with all *mycobacteria*, rely on their thick waxy outer membrane for survival, as well as aiding in their ability to cause infection in their host. These lipids are responsible for ~60% of the dry weight of the bacterium and have been shown to be strong modulators of the host immune system. Several species of *Mycobacterium*, such as *Mtb* and *M. leprae,* can contain a specific lipid on their outer membrane known as PGL (Fig. 3). PGL in *Mtb.* (known as PGL-tb) is found only in the West Beijing family of *Mtb* (Fig. 1)

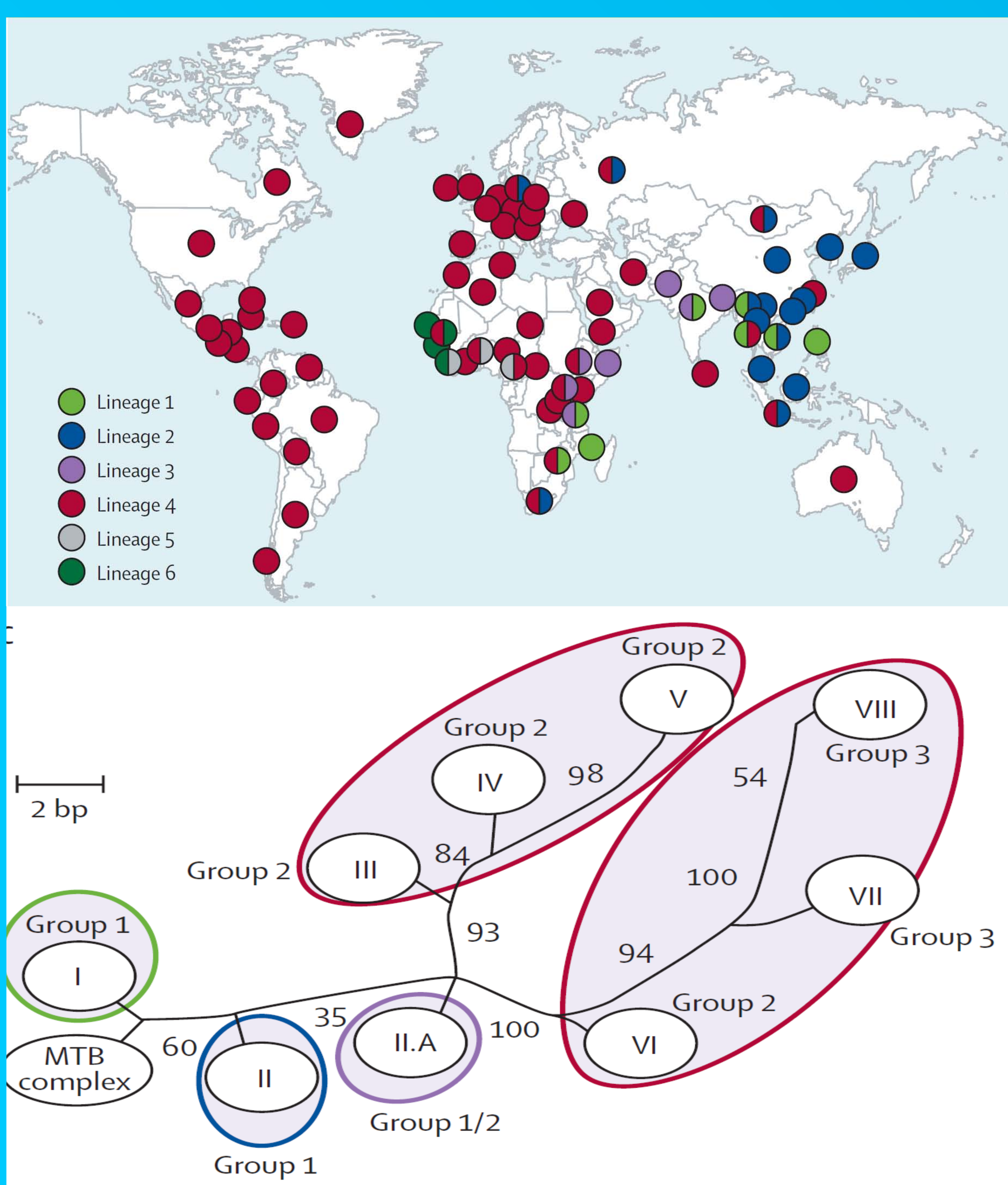


**Figure 1. Phylogenetic tree of *Mtb* and the areas of influence by different clades. The West-Beijing strains (such as HN878) are associated with Lineage #2.** [5]

## *Mycobacterial* Cell Wall

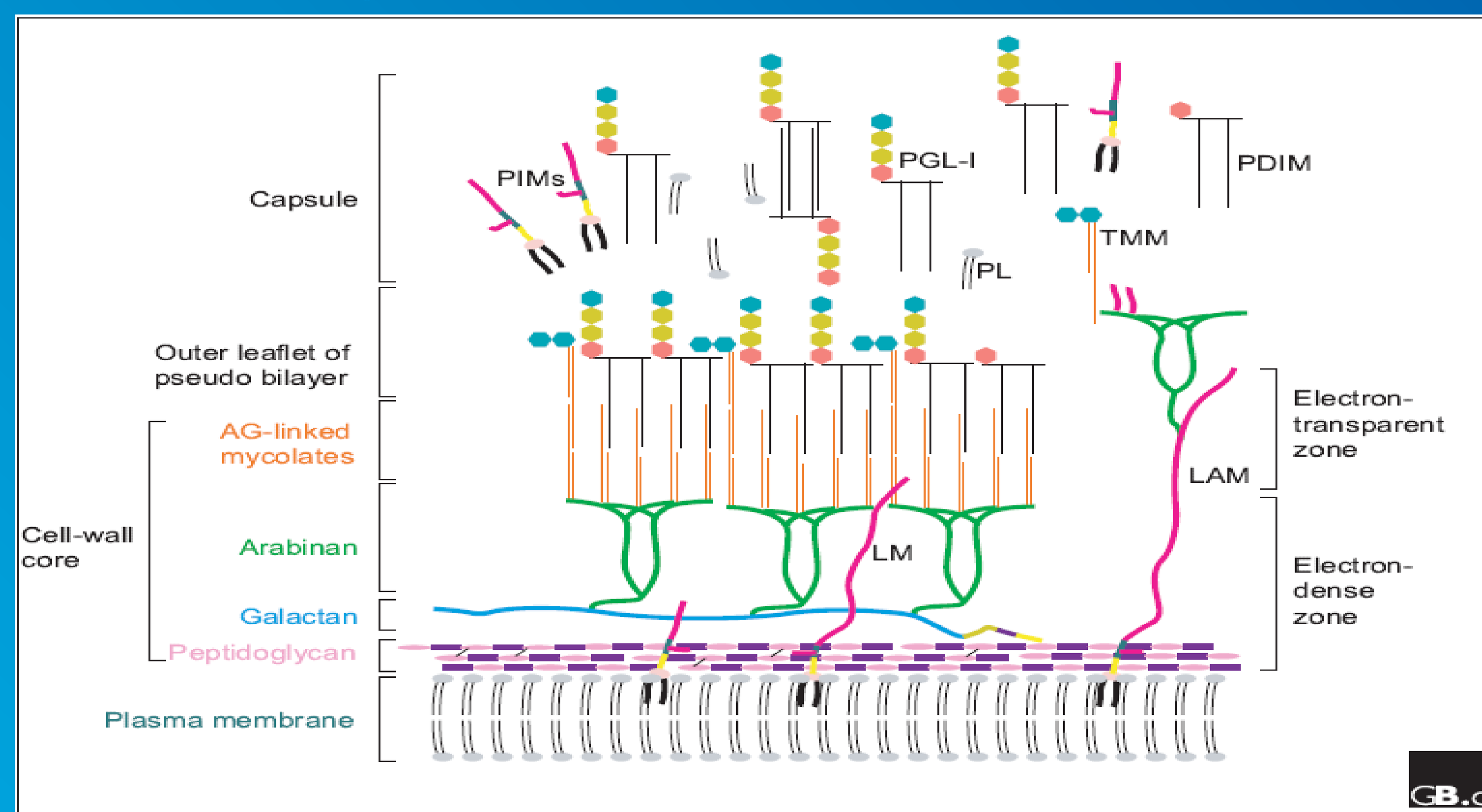


**Figure 2. Example of *M. leprae* cellular envelope and several lipids found associated with it.** [4]

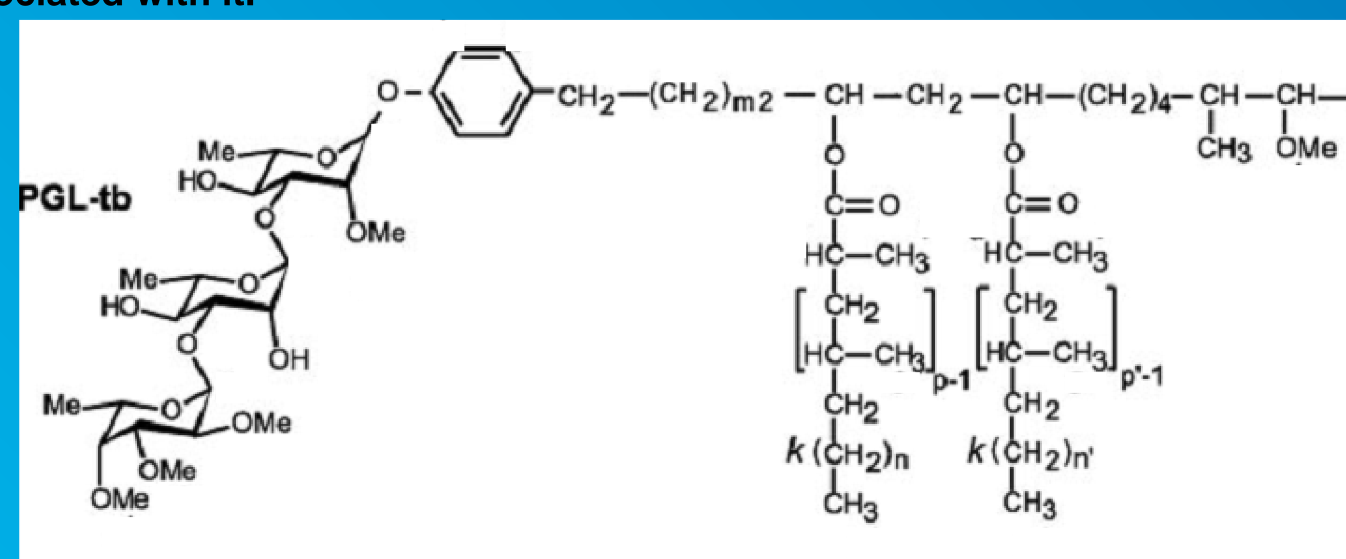


**Figure 3. Phenolic glycolipid found in *Mycobacterium tuberculosis*.**[3]

## Materials and Methods

•1. γ-irradiated Mtb strain HN878 cells were lyophilized and de-lipidated via 1:2 $CHCl_3$/MeOH.
•2. The organic extract was dried, resuspended in 1:2 $CHCl_3$/MeOH, and analyzed via analytical TLC.
•3. A Folch Wash, which is a technique using various amounts of water to remove polar glycolipids was carried out, and the aqueous phase was removed.
•4. Preparatory TLCs utilizing a 95:5 $CHCl_3$/MeOH solvent system to separate lipids.
•5. Plates were sprayed with $CuSO_4$ and/or α-naphthol to detect organic compounds and sugars. These were then charred to develop.
•6. Plates viewed under UV light, PGL bands outlined, and plates scraped for purification of PGL.
•7. Lipids were extracted from the silica using either: 100% $CHCl_3$, diethyl ether, and 1:1 $CHCl_3$/ n-butanol.
•8. Extracted lipids were analyzed by analytical TLC.
•9. A second purification step was performed using similar methods.
•10. Lipids were extracted from silica using diethyl ether and analyzed via analytical TLC.

**Figure 4. Preparatory TLC in 90:10 $CHCl_3$/MeOH solvent system and sprayed with $CuSO_4$. The top arrow depicts the *M.* leprae control and what we believe to be the PGL band. This band was viewed under UV and outlined. The bottom arrow shows the lines where the PGL control and whole lipid were applied. Center silica was not sprayed since this is where our lipids of interest reside.**

**Figure 5. Analysis of whole lipid via an analytical TLC stained with $CuSO_4$.**

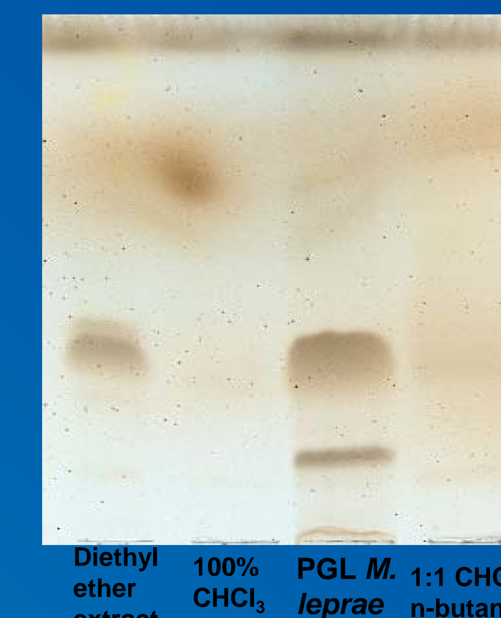


**Figure 6. Analytical TLC showing PGL extraction using various solvents. Arrow depicts putative PGL band. This plate was resolved in 95:5 $CHCl_3$/MeOH.**

**Figure 7. Analytical TLC showing extraction of silica with diethyl ether from second purification.**

## Results

The results from our experiments from the first preparation are encouraging. As seen in figure 6, diethyl ether seemed to extract PGL with relatively low amounts of contamination. The diethyl ether extraction from the second preparation also showed PGL bands, of relative purity figure 7.

## Discussion

We carried out two lipid preparations in this project. In the first one we extracted γ-irradiated *Mtb* HN878 cells with 1:2 $CHCl_3$/MeOH and analyzed the total lipids with TLC. During analysis we noticed one of the bands migrated the same distance as a major PGL control band. We used preparatory TLCs to separate whole lipid extract components, and to specifically isolate the PGL band. We then used various solvents to extract PGL from the silica: 100% $CHCl_3$, 1:1 $CHCl_3$/n-butanol, and diethyl ether. Diethyl ether seemed to be the optimal solvent to extract PGL because it reproduced the intense band we observe as PGL with little lipid contamination. During our second preparation, we attempted to isolate and purify PGL using refined lipid isolation techniques. We found that our relatively pure product, extracted with diethyl ether, lined up with the PGL control.

When observed on many of the analytical TLCs there are always two bands in the control. We assume that one or both of these two major bands must be PGL. The intensity of the upper band leads us to conclude that it is PGL. We think the other band may be a breakdown product of the PGL control or a difficult contaminant to remove.

## Future Direction

Additional work will involve a second preparative TLC run of our product for further purification, and quality control analysis of purified products via 2D-TLC, MALDI-TOF and H-NMR.
Once we are able to purify PGL, we will then begin analyzing the effects of naïve and *Mtb* infected macrophages and the effects of PGL during the pro-inflammatory response.

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