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Comparitive analysis of the lipidomic profile of *Mycobacterium tuberculosis* H37Rv in different carbon sources.

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ABSTRACT

Mycobacterium tuberculosis is well adapted to the most challenging environmental conditions that are present inside macrophages ranging from nutrient scarce medium, to the immunological evasions by the host. Amid all these challenges it not only saves itself but also modifies the host environment as per its needs. The utilization of unusual fatty acids is one such example. Fatty acid utilization by Mtb not only provides energy but also helps in the synthesis of several unique lipids. Hence we have studied the impact of different carbon sources on the lipidomic profile of Mycobacterium tuberculosisand have found that out of the three carbon sources i.e. Cholesterol, Glycerol and Acetate, Cholesterol modifies the lipids of Mtb significantly.

KEYWORDS

Introduction

Mycobacterium tuberculosis, one of the most prevalent causes of death worldwide, is a facultative intracellular parasite that invades and persists within the macrophages. In addition, this pathogen needs multidrug therapy to be effectively treated. These properties have been linked with the presence of high lipid content in its cell envelope with ~30% of its genes devoted to lipid synthesis / metabolism[1]. Wide range of lipids are present in the cell wall of Mtb ranging from Mycolic acids, TrehalsoeDimycolates (TDM), Trehalosemonomycolates (TMM), PhthiocerolDimycoserosates (PDIM), Phenolic Glycolipids (PGL's),Sulfolipids etc. The lipidomic profile of the bacterium is very dynamic in nature and changes its composition as per the bacterium's requirement. As we know that the MTB resides in the macrophages of the human host where the nutrient availability is very scarce and hence it utilizes several unsusual compounds present inside the macrophages such as fatty acids. Availability of different carbon sources inside the macrophages may have a direct impact on the composition of cell wall of the Mtb which is found to vary in different physiological stresses. This dynamism of the cell wall of the Mtb is responsible for combating various immunological activities of the human host.Therfore in this study we have tried to find out the effect of three different carbon sources i.e. cholesterol, glycerol and acetate onto the lipidomic profile of *Mycobacterium tuberculosis*using gas chromatography.

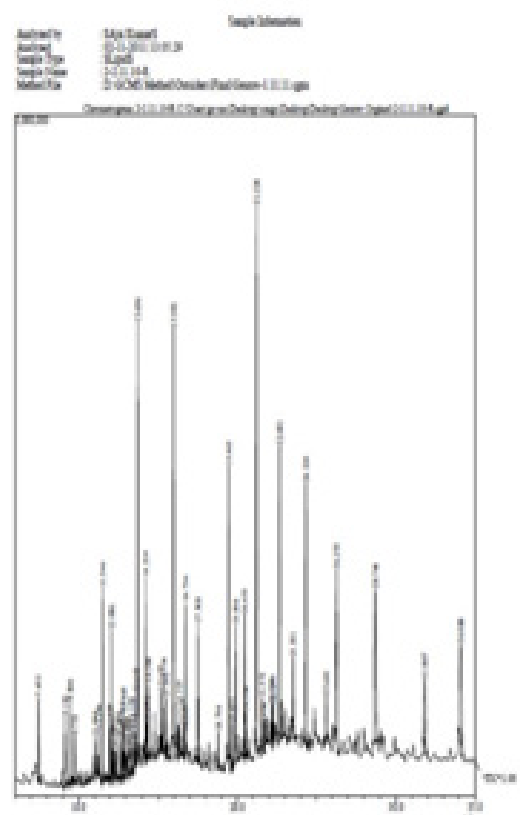
Material and Methods

Bacterial strains and growth conditions: For growth on different carbon sourcesviz a viz cholesterol, glycerol and acetate , *M. tuberculosis* was grown in minimal medium (0.5 g/liter asparagine, 1 g/liter KH2PO4, 2.5 g/liter Na_2HPO_4, 50 mg/liter ferric ammonium citrate, 0.5 g/liter $MgSO_4{\cdot}7H_2O$, 0.5 mg/liter $CaCl_2$, and 0.1 mg/liter $ZnSO_4$) containing either 0.1% glycerol (vol/vol),0.1% acetate (wt/vol), 0.01% cholesterol (wt/vol) at 37°C. Before addition, cholesterol was dissolved at 100 mg/ml in a solution of tyloxapol:ethanol (1:1) at 90°C.

Extraction of Mycobacterial lipids: Cultures were grown until OD reaches 0.4-0.6.Cultures were then autoclaved and*M. tuberculosis* H37Rv grown in different carbon sources separately was subjected to lipid extraction by Bligh and Dyer[2]. In brief, lipids were extracted first by subjecting the three different pellets to $CHCl_3/CH_3OH$ (1:2, v/v) solution with proper mixing for two days at room temperature and then in $CHCl_3/CH_3OH$ (2:1, v/v). The two organic phases thus obtained were concentrated, washed thrice with distilled water and dried to get crude lipid extracts.

Derivatization of extracted lipids: Lipids extracted above were mixed with 30% (w/v) methanolic potassium hydroxide (2 ml) and toluene (2 ml) and heated at 100°C for 1 h. Afterwards the samples were dried with Nitrogen[3]. Trimethylsilylderivatives were formed by adding BSTFA (50 µl) and pyridine (10 ml) to the dried samples and heatingat 80°C for 15 min[4]. Pyridine was removed by using a stream of nitrogen and finally hexane (2 ml) was added to each sample.

Gas Chromatography: Gas Chromatography for the three different samples was performed on GCMS QP 2010 with a flow rate of 10 & held for 35 minutes at 240°C.



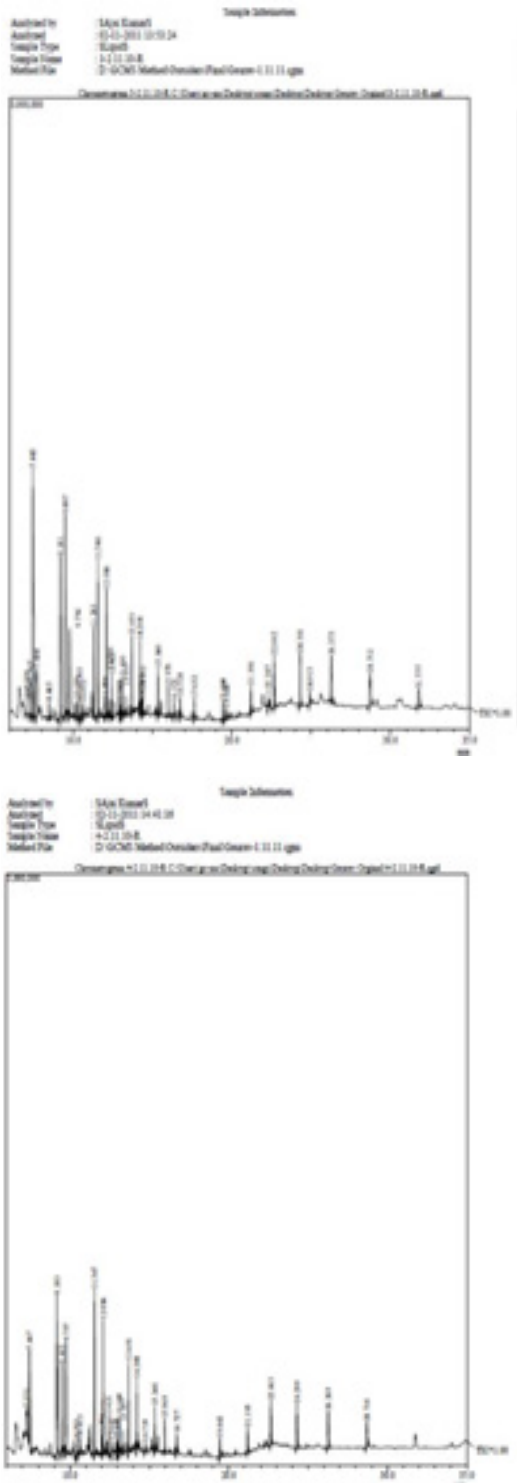


Figure 1:- Gas Chromatogram obtained for the lipidomic profile of the *Mycobacterium tuberculosis* grown in the presence of (a) Cholesterol (b) Glycerol (c) Acetate.

As per the results obtained after the gas chromatography analyses of the lipid profile of Mtb grown in three different media i.e. Cholesterol, Glycerol and propionate. It is clear that the most visible change in the lipidomic profile was seen when the Mtb was grown in the presence of cholesterol. The reason for the most stringent change can be attributed to the breakdown products that are obtained after cholesterol degradation inside *Mycobacterium tuberculosis* H37Rv. Cholesterol degradation inside Mtb produces a large amount of acetyl Coa and Propionyl CoA. This large pool of acetyl CoA and propionyl CoA is diverted towards the production of lipids of Mycobacterium tuberculosis[5]. These two moeities i.e. Acetyl CoA and Propionyl CoA serve as a precursor for various virulence lipids of *Mycobacterium tuberculosis* such as Mycolic acids, Phthiocerol Dimycoserosates, Sulfolipids etc. Hence the changes in the lipidomic profile of Mtb in the presence of cholesterol can be attributed to the production of acetyl CoA and Propionyl CoA.

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