Insoluble Fractions

The insoluble fractions form backbone of the cell wall of the *Mycobacterium tuberculosis* and is made up of peptidoglycan, arabinogalactan, mycolic acid.

Peptidoglycan: There are three features in the peptidoglycan of *Mycobacterium tuberculosis* that distinguishes it from the *E. coli*. Apart from the normal alternate repeating units of N- acetyl glucosamine and N-acetyl muramic acid as seen in *E. coli*, *Mycobacterium tuberculosis* peptidoglycan have an extra element that consist of N-glycolylmuramic acid which is formed by the acetylation of muramic acid of the peptidoglycan with the glycolic acid[2]. Cross linking peptide that links dimer of N-acetylglucosamine and N acetyl muramic acid in *E. coli* consist of Ala-Glu-DAP-Ala linked via Ala-DAP of the repeating units, while in *Mycobacterium tuberculosis*it consist mostly of DAP-DAP linkages[3]. The degree of cross linking of the repeating units in *Mycobacterium tuberculosis*is about two and half times higher than the *E. coli*. This unusual cross linking in the peptidoglycan of the *Mycobacterium tuberculosis* helps in its survival against the conventional proteases

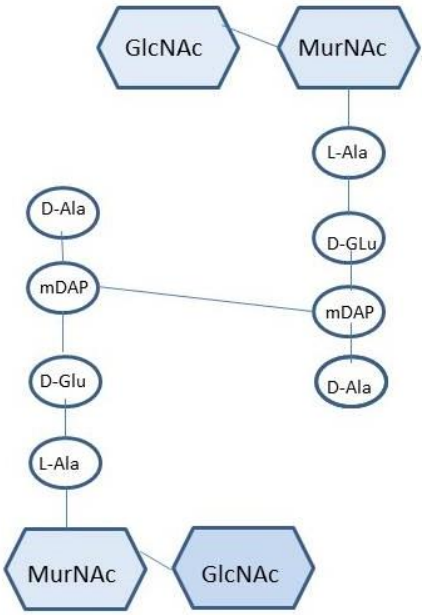

Figure 2: Representative diagram of Peptidoglycan

Arabinogalactan

The arabinogalactan is sandwiched between peptidoglycan on one side and mycolic acid on the other and hence forms PAM(peptidoglycan-arabinogalactan-mycolic acid) complex. It is made up of two components i.e. arabinan and galactan that are attached to each other via a linker molecule. Arabinans are linked via an Araf-α(1→5)-Galfglycosidic bond togalactans which is a polysaccharide consisting of approximately 30 β(1→5) and β(1→6) Galf residues[4].Arabinan and galactan together forms arabinogalactan moiety.

Mycolic Acid

Mycolic Acids are essential for the survival of *Mycobacterium tuberculosis* and consist of about 32% of the dry weight of *Mycobacterium tuberculosis*.They are β Hydroxy fatty acids consisting of an alkyl side chain with total number of carbon ranging from 60-90. There are three types of mycolic acids that are found in *Mycobacterium tuberculosis* i.e. alpha, methoxy and keto mycolic acids. Out of all three, Alpha mycolic acids are the most abundant. Mycolic acids are synthesized by the fatty acid synthase complex of *Mycobacterium tuberculosis* consisting of two system FAS I(multienzyme complex) and FAS II. FAS I enzyme is responsible for the synthesis of C20 and C26 fatty acids that are attached to CoA. Further elongation of these C20 and C26 fatty acids is carried out by FAS II system that results in the formation of meromycolate. Several other enzymes are responsible for the differentiation into α, methoxy and keto mycolic acids which are beyond the scope of this review

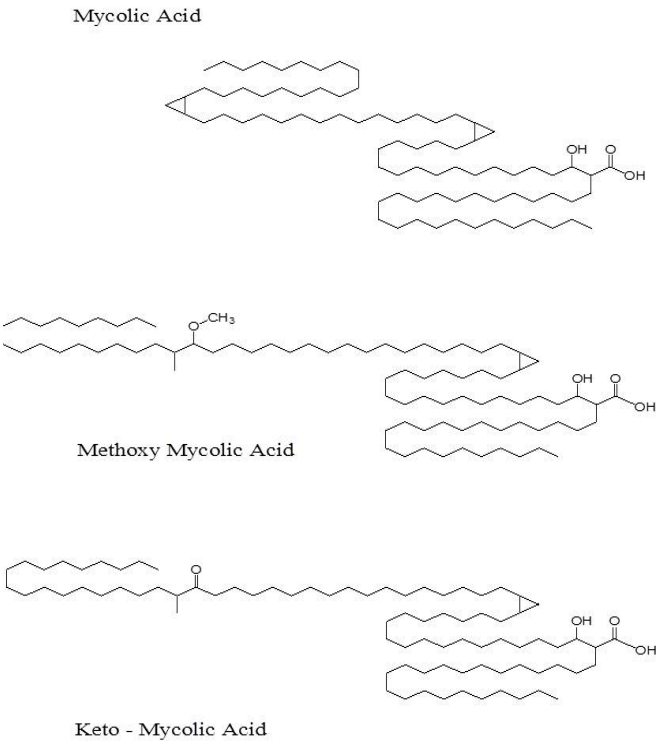

Figure 3: Representative diagram of the structure of Mycolic acid from *Mycobacterium tuberculosis*

2. Soluble Lipids

PDIM(phthioceroldimycoserosates) Phthiocerol and phenolphthioceroldimycocerosateses are a group of free lipids that are present in the upper layer of the cell wall of *Mycobacterium tuberculosis*. Mycobacterium mutant lacking genes that are responsible for the synthesis of PDIM was shown severely attenuated[5]. It is an important virulence factor of *Mycobacterium tuberculosis*which isrequired for the maintenance of cell wall permeability and it is specific to pathogenic mycobacteria[6]. Structurally it is a mixture of long-chain β-diols (C33–C41) esterified by the multimethyl-branched mycocerosic or phthioceranic acids (C27–C34).mas enzyme is responsible for the synthesis of mycocerosic acid which comprises ofmethylmalonyl CoA's(MMCOA) repeated additions[6]. However,Phthiocerol synthesis requiresenzymes encoded by different genes i.e. ppsA-E.mmpl are the set of genes required for the translocation of lipids to their target sites. Mmpl7 is responsible for the transport of PDIM[7].

Sulfolipids: Sulfolipids are sulfatedtrehalose esters that are acylated with three or four acyl groups consisting of one saturated fatty acid i.e. either palmitic acid or stearic acid at the 2- carbon position and a combination of the hepta- and octamethyl- branched phthioceranic and hydroxyphthioceranic acids (C31–C46) at the 3- ,6- and 60-positions.*pks2* and *mmpl8* are the two most important enzymes thathelp in the synthesis of sulfolipid in *Mycobacterium tuberculosis*[8,9]. Out of the *Mycobacterium tuberculosis*complex they are found only in *Mycobacterium tuberculosis* and hence play an important role in the virulence of *Mycobacterium tuberculosis* primarily at the early stage of infection. They also inhibit oxidative phosphorylation in mitochondria[10] and inhibits the fusion of lysosome with the phagosome thereby modulating the immune response of the human host[11].
It has already been proved that the influx of propionyl CoAresults in the increased production of the sulfolipids and PDIM. Therefore, when *Mycobacterium tuberculosis*thrives on the fatty acids as a carbon source, synthesis of PDIM and sulfolipid acts as a sink for the toxicpropionyl CoA which is aprecursor for the biosynthesis of sulfolipid and PDIM[12]. So it performs dual function of a virulence factors as well as a savior for *Mycobacterium tuberculosis* from the toxicpropionyl CoA.

Cord factor- also called as trehalose 6,6dimycolate is present at an higher concentration as compared to PDIM and sulfolipids. It is found in every species of mycobacteria and play an important role in granuloma formation inside the host. It also inhibits fusion of phagosome with lysosome and thereby prevents the acidification of the phagosome. *Mycobacterium tuberculosis* devoid of its lipidomic armamentarium shows reduced survivability and infection and restores the growth in vitro when injected with the purified TDM[13,14]. It consists of two mycolic acid moieties that are attached to the trehalose sugar via hydroxyl group. Purified TDM when injected with oil droplets induces granuloma formation in mice but fails to do so when injected alone[15].

3. Conclusion

The importance of the lipids in the pathogenesis and survival of *Mycobacterium tuberculosis* can be ascertained by the fact that gene responsible for lipidomic function of *Mycobacterium tuberculosis* accounts for 30% of the total genome. Lipid specifically that forms the part of the cell wall of the mycobacteria are actively involved in the pathogenesis, infection & virulence and forms an indispensable part of the life cycle of the bacteria. They are even responsible for modulating the host immune responses. In many of the cases their absence renders the bacteria attenuated. This phenomenon has attracted scientists to target the various enzymes that are involved in the biosynthesis of these lipids. Importance of lipids as drug targets can be understood by the fact that Isoniazid, a mandatory first line drug, targets the lipid biosynthesis machinery. To effectively manage the disease we need to target those domains that would make thisbacteria most susceptible. Lipidomic machinery of the *Mycobacterium tuberculosis* opens new vistas in the field of anti-mycobacterial drug targets that can prove to be achilles heel in case of tuberculosis.

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