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Review

# The *Mycobacterium tuberculosis*: A Silent Killer

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**Abstract:** *Mycobacterium tuberculosis* is the agent of tuberculosis, one of the most important infectious diseases in the world. This microorganism stands out from other bacteria, not only for its extremely high infection capacity, but also for its cellular characteristics that include an extremely resistant and hydrophobic cellular parade the passage of antibiotics. An incredible ability to adapt to adverse conditions inside the host as well as its vast arsenal of virulence factors that allow its survival within the inhospitable environment within the macrophage can be highlighted. This review aims to discuss several aspects of MTB microbiology, genetics, and physiology. We will address in this review details of the metabolism of MTB that allows it to replicate in the active phase and remain viable during latency, as well as the characteristics of its cell wall that contribute to the blockade of the immune response and its resistance to antibiotics.

**Keywords:** tuberculosis; *Mycobacterium*; physiology; mycolic acids; cell wall

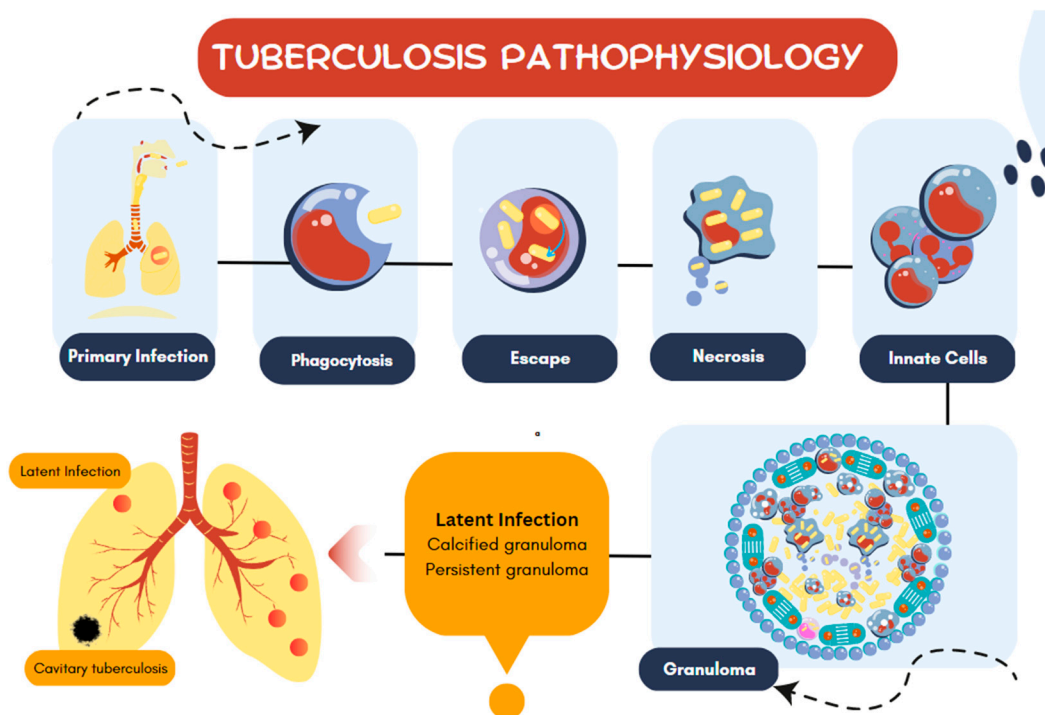
## 1. Introduction

The World Health Organization (WHO) estimates that one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB), the agent of tuberculosis (TB), and that about 5 to 10% of those infected will develop active disease at some stage of your life. MTB was first described in 1882 by Robert Koch, thereafter, being commonly referred to as Koch's bacilli in honor of its discoverer. Since its discovery, the bacillus has been the target of several studies and campaigns aimed at its eradication [1–3].

MTB is transmitted through the inhalation of aerosols emitted by patients in the active phase of the disease. Upon inhaling MTB is phagocytosed by alveolar macrophages, yet other cells such as dendritic cells and neutrophils also can phagocytose MTB, triggering an immune response that recruits more immune cells to the site. Finally, with the development of the progressive immune response, a local lesion called granuloma appears. This lesion is a hallmark of TB and although it is not pathognomonic helped in the diagnosis of active disease [4–8].

MTB infects only human hosts, and the infecting dose is extremely low, only 5–10 bacilli can establish the initial infection. In the interior of the macrophage, the MTB is inside of modified phagosomes, to establish the infection and to avoid the immune system the MTB is used of diverse strategies like the inhibition of phagosome maturation, inhibition of the presentation of antigens, among other [4,9].

It is important to note that several people will contact the MTB and will be able to control the spread of the disease due to the action of the immune system. However, these individuals have great potential to have residual bacilli and, therefore, to become ill phagosome throughout their lives. In infected people, the risk of developing active disease increases over time, but in patients with immune deficiencies such as HIV, this risk is even greater, Figure 1 [10–13].



**Figure 1.** Tuberculosis pathophysiology. The pathophysiology of tuberculosis includes phagocytosis of the bacillus by lung macrophages, followed by escape into the cell cytoplasm after preventing lysosomal fusion to the phagosome. Escape can induce cell necrosis, which induces an increase in cellular immune response that culminates in granuloma formation. The granuloma may contain the infection or function as a site for replication of the bacilli in the latent disease.

Although a wide range of efforts have been expended for the diagnosis, prevention, and treatment of tuberculosis in recent decades, tuberculosis still figures as one of the leading causes of death in the world [1,14].

## 2. *Mycobacteria* and *Mycobacterium tuberculosis*: An Interesting Genus

*Mycobacteria* are bacilli, phylogenetically related to actinobacteria, with large amounts of C-G in their DNA. They present an extremely complex cellular envelope, which is characterized by the rich presence of mycolic acids. The *Mycobacterium* genus comprises more than 150 species including *Mycobacterium tuberculosis* Complex (MTBC), *M. avium* Complex (MAC), *M. terrae* Complex, *M. abscessus-chelonae* Complex, *M. simiae* Complex, *M. fortuitum-smegmatis* Group, *M. celatum* Group, *M. xenopi* Group and others non-tuberculous mycobacteria (NTM). *Mycobacteria* are identified by Ziehl-Neelsen staining. They are acid-fast bacteria, which during staining with fuchsin retain this dye even after washing with alcohol-acid due to the characteristics of its cell wall. This cell wall is composed of a layer of peptidoglycan, and a micomembrane composed of mycolic acids, arabinose, and other sugars, which make this membrane highly hydrophobic and provide great protection to the bacillus against external agents such as antibiotics [15–21].

Potentially pathogenic can cause lung disease or even disseminated disease in immunocompromised patients. The MTBC is formed by the species *M. tuberculosis*, *M. canettii*, *M. africanum*, *M. microti* and *M. bovis*, recently was reported more two new species *M. mungi* and *M. orygis*. In addition to two subspecies *M. caprae* and *M. pinnipedii*. The bacteria belonging to the MTBC present about 99.95% of genetic identity and although there is clearly a great similarity in genome level, the members present different preferences regarding the host, pathogenicity, and phenotype [11,22,23].

*M. bovis* can cause disease in humans whose disease cannot be distinguished from that caused by MTB. Although its incidence is low nowadays thanks to the advent of pasteurization, which has

virtually eliminated the main route of transmission, transmission through the ingestion of contaminated milk produced by contaminated cattle, *M. bovis* disease has been increasing in immunocompromised patients, especially those infected with HIV. *M. africanum* is an important agent of TB, accounting for half of the cases in West Africa [24–26].

The *M. Avium* Complex is formed by *M. avium* subsp. *avium* (MAA), *M. avium* subsp. *hominissuis* (MAH), *M. avium* subsp. *paratuberculosis* (MAP), *M. avium* subsp. *silvaticum* (MAS), *M. avium* subsp. *lepraemurinum*, *M. arosiense*, *M. bouchodurhonense*, *M. colombiense* and *M. intracellulare*, which may cause lung disease and cavitary lung disease in immunocompromised patients, but in immunocompetent patients, the disease is rare [27–32].

Other important NTM is the *M. ulcerans*, agent responsible for *Buruli* ulcer. This mycobacterium presents the production of an important cytotoxin, mycolactone, responsible for cellular necrosis and immunomodulation. In addition, the other important pathogenic slow growers are *M. marinum*, *M. haemophilum*, *M. genavese*, *M. kansasii*, *M. malmoense*, *M. simiae*, *M. xenopi* and *M. szulgai* [20,33–37].

Among the species, we must also give relevance to *Mycobacterium leprae*, which is the agent of leprosy. Although the disease is one of the oldest, still today the culture of this bacterium cannot be performed in laboratory media. NTMs are in the environment, mainly soil and water and are classified according to the growth rate in culture. Currently many NTMs have been causing illness in patients who have undergone invasive procedures, such as ophthalmic, aesthetic, and cardiac surgeries, or constant use of injectable drugs. The most frequent NMTs in these cases are the fast-growing *M. fortuitum*, *M. abscessus* and *M. chelonae*. However, the isolation of NTMs requires great care since it may be the result of simple contamination or temporary colonization. Table 1 shows the main groups and complexes of the *Mycobacteriaceae* family and their representatives [33,38,39].

Table 1. *Mycobacteriaceae* groups and complex.

Group or Complex	Principal Species	Pathogenicity and Disease <sup>1</sup>
<i>M. tuberculosis</i> Complex (MTBC)	<i>tuberculosis</i>	Pathogenic/ tuberculosis
	<i>bovis</i>	
	<i>africanum</i>	
<i>M. kansasii</i> Group	<i>kansasii</i> <i>gastri</i>	Pathogenic/ pulmonary and soft tissue
<i>M. avium</i> Complex	<i>avium</i> <i>colombiense</i> <i>intracellulare</i>	Pathogenic/pulmonary in immunocompromised
<i>M. simiae</i> Complex	<i>kubicae</i> <i>florentinum</i>	Generally non-pathogenic
<i>M. celatum</i> Group	<i>xenopi</i> <i>celatum</i>	Pathogenic/pulmonary in immunocompromised
<i>M. terrae</i> Complex	<i>terrae</i> <i>hibernae</i>	Generally non-pathogenic
<i>M. smegmatis</i> Group	<i>smegmatis</i> <i>thermoresistibile</i>	Generally non-pathogenic
<i>M. fortuitum</i> Group	<i>fortuitum</i> <i>peregrinum</i>	Pathogenic/soft tissue

<i>M. abscessus-chelonae</i> Complex	<i>abscessus</i> <i>chelonae</i>	Pathogenic/pulmonary in immunocompromised
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<sup>1</sup> Pathogenicity is considered as the main characteristic, some mycobacteria only cause disease in immunosuppressed patients and therefore were not considered pathogenic.

The history of TB is closely linked to the history of humankind, with signs of TB being found in European and Middle Eastern skeletons of 4000 years until the description of tuberculosis by Hippocrates in ancient Greece. In Europe in the 16th and 17th centuries, TB was one of the main infectious diseases of the time, with thousands of associated deaths<sup>41</sup>. Now is widely accepted that MTB originated in Africa more than 7000 years ago from environmental mycobacteria. With the aid of the establishment of agriculture, livestock, establishment of the civilization and increase of the population density it was possible to select virulent strains and the populations transmitted that[40–44].

It is interesting to note that MTB in the Americas has become a major problem for indigenous communities during colonization by the European peoples. MTB was believed to be a microorganism evolved from *M. bovis* that causes the same human pathology in cattle; yet new studies have shown that the only difference between the members of the *M. tuberculosis* Complex corresponds to the polymorphisms in certain genes, suggesting, therefore, that both bacilli evolved in parallel [45].

**3. *Mycobacteria* and *Mycobacterium tuberculosis*: An Interesting Genus**

MTB is a straight or slightly curved bacillus, immobile and non-sporulating. It has a lipid-rich cell wall, is resistant to alcohol-acid discoloration, and is called alcohol-acid resistant bacilli. MTB exhibits an extremely slow generation time of 20 to 22 hours, nonchromogenic colonies, rugose-like suede appearance, growing between 14 to 28 days at 37 °C in solid medium. In addition, samples from non-sterile sites may affect the bacillus growth. The cell wall of the MTB has as main constituents the mycolic acid, peptidoglycan and arabinogalactans, being covered by polysaccharides and proteins that are not covalently bound. This wall is responsible for an important degree of microorganism resistance to the microbicidal mechanisms present inside the macrophage [46–49].

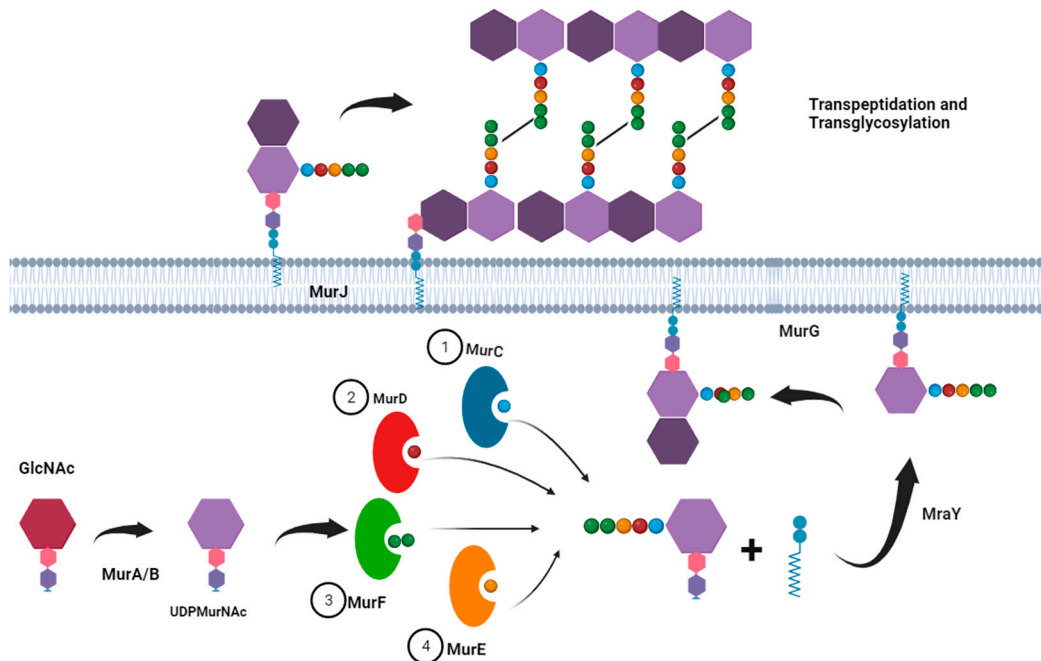
Recent studies of cryomicroscopy allied to tomography suggest that the mycomembrane of MTB, is organized like the external wall of Gram negative and that presents proteins with function of porins. It shows that the cell wall of this important pathogen would be much more complex than it has been described today [50,51].

It is estimated that the MTB genome shelters about 140 proteins in the mycomembrane, none of which have been identified and characterized. This fact is even more striking considering that in *M. smegmatis*; the MspA protein was structurally identified and characterized. MspA consists of the major pore of *M. smegmatis*, and its deletion showed decrease in permeability to glucose, phosphate, metal ions and amino acids. This interesting porin consists of eight monomers, showed the presence of a channel formed by  $\beta$ -sheets, besides a large external protuberance also formed by  $\beta$ -sheets. The core presents 16 aspartic acid residues that generate a highly negative network, which explains the pore preference for cations. However, no similar protein was identified in MTB [52–58].

The MTB peptidoglycan consists of repeats of the disaccharides N-acetylglucosamine and N-acetylmuramic acid (NAG-NAM), which are produced in the cytoplasm from lipid precursors. The precursors receive the addition of amino acids in their chains, through the Mur proteins, which constitute in ATP-dependent ligases. The MTB peptidoglycan is highly interlaced, with cross-links 3-3 instead of the traditional 4-3, in addition, they have different alterations such as NAM glycosylation, D-Glu amidation and mesodiaminopimelic acid (mDAP) in lateral chains. It is interesting to note that imidation may inhibit the recognition of MTB by the immune system; however, the presence of glycosylation induces the production of inflammatory cytokines by the macrophage. In addition, there is the action of hydrolases that will act on the anterior peptidoglycan, opening space for the insertion of the new peptide, whereas penicillin-binding proteins (PBPs), such as PonA1 and PonA2, incorporate the units in the structure. Finally, PBPA, PBPB, LdtA and LdtB are transpeptidases that promote crosslinks between the nascent structures [59–62].



MTB shows the MurA, MurB, MurC, MurD, MurE and MurF proteins and the product of these proteins are exported with the action of the transmembrane protein MviN. The proteins MurI, DapF and DdlA are responsible to produce the substrates for the Mur proteins Figure 2 [62–66].



**Figure 2.** MTB peptidoglycan synthesis. MTB peptidoglycan is synthesized from precursor sugars present in the bacillus cytoplasm. Mur enzymes sequentially add five amino acids to the NAM, then the NAM is inserted into the inner leaf of the cytoplasmic membrane. The MurG protein links NAM to NAG and MurJ transports the block to the outer layer of the plasma membrane. In the periplasm transglycosylase proteins and transpeptidases such as PBPs and Pon will link NAG-NAM building blocks to form peptidoglycan layers.

The arabinogalactan present in the cell wall of the MTB is composed of a galactan formed by the repetition of the disaccharide of 6-d-Galf $\beta$ 1-5-d-Galf $\beta$ , being formed by the action of galactofuranosyl transferases GlfT1 and GlfT2. Like NAM, galactan is modified by the addition of long arabinan polymers which are produced by DprE1, DprE2, AftA, AftB, EmbA and EmbB and their ends are branched by AftC, AftD and Rv1459. Arabinans may also receive modifications as addition of succinyl or non-N-acetylated galactosamines (GalN), which is found in many pathogenic mycobacteria and appears to be important for an efficient infection. Finally, the arabinans are bound to mycolic acids, which are large hydroxylated fatty acids of branched chains, being covalently attached to the cell wall, forming the mycomembrane, Figure 3 [67–70].

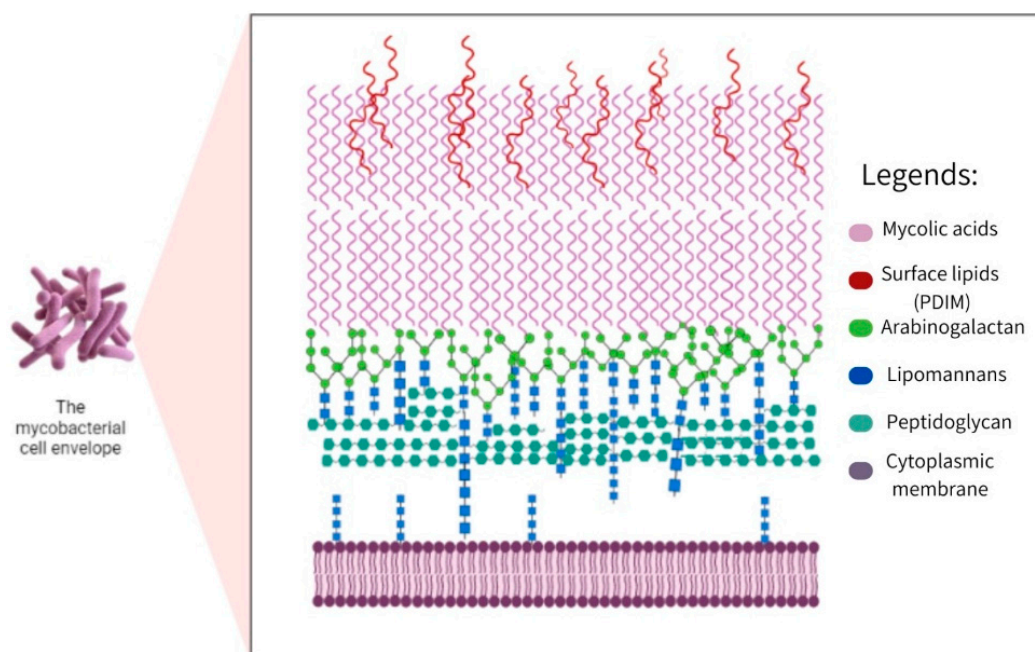
The FASI (FAS I enzyme) and FAS2 enzyme complexes (composed of MabA, HadABC, InhA, KasAB and an isomerase) that promote the union and branching of two fatty acids form mycolic acids. Subsequently undergo maturation promoted by a series of enzymes which results in three different molecules at the end:  $\alpha$ -meroacids, methoxy-meroacids and ketomeroacids, all three being important for virulence. Other lipids that are components of the cell wall of mycobacteria are trehalose dimycolate (TDM) that promotes the cord factor (the cord factor consists of serpentine MTB clusters observed in the lamina stained by Ziehl Neelsen), glycerol monomycolate, glucose monomycolate (GMM), triacyl-trehalose (TAT), diacyl trehalose (DAT), sulfolipid (SLs), pentacyl trehalose (PAT), mannosyl- $\beta$ -1-phosphomycocetos, phenolic glycolipids (PGLs) and phthiocerol dimycocetate [71–74].

Most genes involved in lipid synthesis are essential for MTB survival. Mycobacterial Membrane Proteins Large (MmpLs) transports the mycolic acids produced. MmpL are members of the superfamily Resistance Nodulation Division (RND). MTB has fifteen genes coding for RND-type transporters, 13 of which are from MmpL proteins. In addition to the transport of lipids, these

transporters have also been involved in the transportation of drugs, the acquisition of iron and the export of siderophores [75–82].

The formation of the MTB cell wall is an extremely complex process and requires several post-translational transformations, which include phosphorylation, pupilation which is a process of addition of ubiquitin-like proteins, and which direct the proteins for proteolysis through proteosomes. Complex regulation of cell wall formation allows MTB to regulate its growth under different conditions within the host, even being a likely mechanism of persistent infection. Therefore, the MTB cell wall has several molecules that induce the immune response [83–88].

MTB presents for cell division the FtsZ protein, a GTPase that forms the central ring of the division and recruits the other proteins involved in the process; FtsE, a protein like ABC type transporters, but without known function. In addition, FtsK, a translocase DNA responsible for chromosomal segregation and septal formation; FtsW, responsible to produce peptide glycol in the septum and translocation of the peptide. A finally FtsI, acts together with FtsW promoting peptidoglycan transpeptidation in the septum; AmiC, an amidase responsible for septal degradation for cell separation and EnvC, also involved in cell separation through septal degradation. In relation to the division, the MTB divides through the elongation and subsequent division of the mother cell into two daughter cells, this division occurring asymmetrically and generating daughter cells of different sizes [89–96].



**Figure 3.** MTB mycomembrane. The MTB mycomembrane is a complex association of several molecules. The first layer is composed of the phospholipid bilayer of the cytoplasmic membrane, followed by a small periplasm. Also in the periplasm is a thin layer of peptidoglycan. Associated with the plasma membrane are several liporabinogalactans that branch with arabinogalactans. Mycolic acids and surface lipids are also linked to arabinogalactans, in addition to the presence of proteins such as the 85B antigen.

#### 4. A Complex Metabolism for Survive and Infect

MTB is a prototrophic bacterium that can synthesize amino acids, vitamins, and cofactors. It presents a complete set of genes involved in the metabolism of fatty acids, tricarboxylic acid pathway and pentose phosphate pathway. In addition, MTB have enzymes that bind the cycle of tricarboxylic acids and the glycolytic pathway. Studies show that MTB can grow rapidly in the first three weeks of infection, reaching its plateau after 2 to 3 weeks after establishment of a Th1-type immune response and increased interferon- $\gamma$  levels. However, auxotrophic mutants for proline, leucine, lysine, tryptophan, purines, or magnesium transport deficiency related to the *mgt* gene, present growth

deficits in the early stages. Some genes that code for enzymes involved in lipid metabolism, such as isocitrate lyase, phospholipase C and *pcaA*, when disrupted and mutated, cause defects in MTB persistence in the late stages of growth, with most of them showing no sign of active disease [97–101].

Cholesterol is an important source of energy for MTB, in addition to being used for the synthesis of lipids linked to virulence. During latent infection, mainly, cholesterol provides many of the products needed for survival for long periods. The metabolism of cholesterol requires the activity of many oxygenases and is probably an explanation of why MTB would infect the lungs, where large concentrations of oxygen are found. The *mce 4* operon has been shown to be responsible for encoding a cholesterol transport system and host membrane lipid uptake, in addition to participating in energy pathways from these lipids, which allows the infection and permanence of the bacilli by long periods of time [102–112].

The MTB to survive inside the host needs carbon and nitrogen sources and for this, it explores several metabolic pathways for these nutrients to be obtained. Carbon sources inside the macrophage are reduced when compared to other environments of the organism, inside the macrophage the MTB increases the expression of isocitrate lyase, which acts on the transformation of acetyl-CoA into carbohydrates through gluconeogenesis. In the macrophage, glucose availability is also one of the critical points for the establishment of MTB, for this we note an increase in the production of enzymes involved with  $\beta$ -oxidation and gluconeogenesis that allows the use of abundant fatty acids in the cellular interior. Phosphate is important for processes of obtaining energy, producing membrane phospholipids, nucleic acids, and many other cellular reactions [98,112–116].

MTB can use ammonia as a source of nitrogen, which is incorporated into glutamate, and glutamine, this process is catalyzed by glutamine synthetase and oxoglutarate synthetase. The bacillus can still grow with  $\text{NO}_3^-$  as the only source of carbon, or even use nitrates that are transported by importers during hypoxia to serve as final electron acceptor. Nitrites generated in the cell interior can be exported out of the cell through exporters. It is interesting to note that in MTB there are several ways to capture and use organic sources of nitrogen, mainly amino acids, the most important being aspartate, asparagine, glutamate, and glutamine that will undergo deamination processes by specific enzymes. Finally, urea can be used as a source of nitrogen, albeit with less efficiency than other organic and inorganic sources. Sulfur in turn is important for the initial process of protein synthesis and for maintaining a redox environment. Therefore, these metabolic systems in MTB present several proteins related to the detection of critical concentrations, capture and transformation of organic and inorganic sources in the molecules of interest [114,117–124].

Among other nutritional requirements, MTB needs the presence of vitamins and other co-factors for survival. For example, many MTB enzymes involved in the synthesis of fatty acids and other lipids are biotin-dependent, so MTB has several genes related to the de novo production of biotin. Another essential cofactor for MTB is nicotinamide adenine dinucleotide (NAD), which is important for various redox reactions, reactions linked to energetic metabolism processes, cobalamin synthesis, and DNA ligase activity. In this way, the MTB can carry out the synthesis of new or recycle from external sources [125,126].

The uptake of metals is an important aspect for the survival of any microorganism, in MTB in addition to the transporters there are the production of siderophores, which are carboxymycobactins produced and exported to the extracellular medium. In the extracellular medium, these molecules bind to non-heme iron and other metals, are internalized by specific transporters that allow the use of these metals, while the less polar mycobactins are associated with the cell and sequester iron. Carboxymycobactins can sequester the iron contained in transferrin and ferritin from the host and IrtAB ABC transporter is responsible for the translocation of these siderophores through the inner membrane. Other essential metals for MTB are zinc, manganese, and cobalt, which are used as cofactors to produce various molecules such as vitamin B12 or even act in response to oxidative stress. MTB presents sensors capable of identifying the levels of these metals in the cellular interior, in addition to the presence of transporters that capture these important metals. It is interesting to note that the host organism often responds by decreasing the concentration of these metals through the action of chelating proteins such as calprotectin [127,128].



Finally, in terms of metabolism, one important point is the oxidative phosphorylation, which allows MTB to produce huge amounts of ATP required for several essential cellular functions. The complex responsible to produce ATP is the F1Fo ATPase synthase, a big complex formed by 10 subunits, and produces ATP utilizing a proton motive force. This complex is closely linked to electron transport chain, responsible for generating a gradient of the protons in the periplasm and recycle donors and acceptors of electrons. Due to its importance for the physiology of MTB, ATPase synthase is an important target for drug development. The new option for the treatment of TB consisted of the inhibitor of ATPase synthase bedaquiline [50,129–132].

**Table 2.** Nutritional Source Table and Metabolism of MTB.

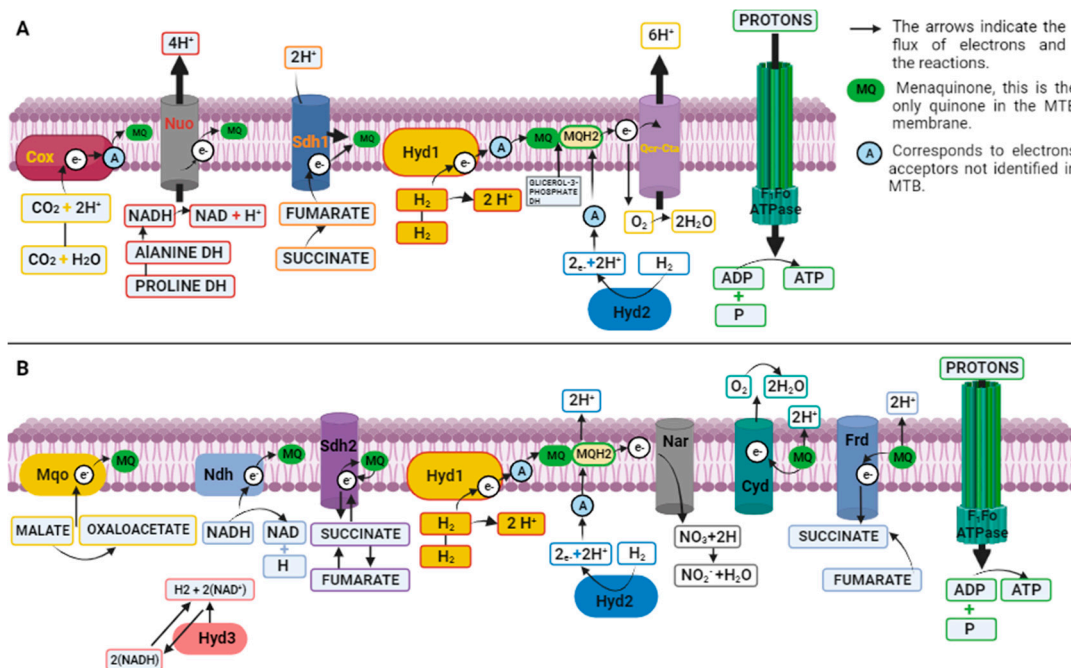
Nutrient/ Metabolism	Gene	Compound	Keeg Code
Ammonium/ Nitrogen	<i>Rv1737c, Rrv2329c, Rv0261c, Rv0267, Rv1161, Rv1736c, Rv1162, Rv1164, Rv0252, Rv0253, Rv0021c, Rv2781c, Rv2476c, Rv2220, Rv2222c, Rv2860c, Rv1878, Rv3859c, Rv3858c, Rv3588c, Rv1284 and Rv3273</i>	C00011, C00014, C00025, C00058, C00064, C00088, C00169, C00192, C00244, C00288, C00488, C00533, C00697, C00726, C00887, C01417, C01563, C05361 and C06058	Mtu00910
Glucose Carbohydrate/ Carbon and energy	<i>Rv0650, Rv0946c, Rv2029c, Rv3010c, Rv1099c, Rv0363c, Rv1438, Rv1436, Rv1437, Rv0489, Rv1023, Rv1617, Rv1127c, Rv2241, Rv2215, Rv0462, Rv2455c, Rv2454c, Rv0761c, Rv1862, Rv0162c, Rv1530, Rv3045, Rv0768, Rv0147, Rv0223c, Rv0458, Rv3667, Rv3068c, Rv2702 and Rv0211</i>	C00022, C00024, C00031, C00033, C00036, C00068, C00074, C00084, C00103, C00111, C00118, C00186, C00197, C00221, C00236, C00267, C00469, C00631, C00668, C01159, C01172, C01451, C05125, C05345, C05378, C06186, C06187, C06188, C15972, C15973 and C16255	Mtu00010
Within the macrophage MTB increases the expression of isocitrate lyase, which acts in the transformation of acetyl-CoA into carbohydrates through gluconeogenesis is	<i>Rv0889c, Rv0896, Rv1131, Rv1475c, Rv0066c, Rv3339c, Rv1248c, Rv0462, Rv2455c, Rv2454c, Rv0952, Rv0951, Rv3318, Rv0248c, Rv3319, Rv0247c, Rv3316, Rv3317, Rv1552, Rv1553, Rv1554, Rv1555, Rv1098c, Rv1240, Rv2852c, Rv2967c, Rv0211, Rv2241 and Rv2215</i>	C00022, C00024, C00026, C00036, C00042, C00068, C00074, C00091, C00122, C00149, C00158, C00311, C00417, C05125, C05379, C05381, C15972, C15973, C16254 and C16255	Mtu00020
Phosphate/ Energy source or phospholipid	<i>Rv0946c, Rv1121, Rv1447c, Rv1445c, Rv1122, Rv1844c, Rv1408, Rv1449c, Rv1448c, Rv2465c, Rv0478, Rv2436,</i>	C00022, C00031, C00117, C00118, C00119, C00121, C00197, C00198, C00199, C00204, C00221, C00231,	Mtu00030

biosynthesis	<i>Rv3068c, Rv1017c, Rv0363c, Rv1099c, Rv2029c, and Rv3010c</i>	C00257, C00258, C00279, C00345, C00577, C00620, C00631, C00668, 00672, C00673, C01151, C01172, C01182, C01218, C01236, C01801, C03752, C04442, C05345, C05378, C05382, C06019, C06473 and C20589.	
Sulfur/ It is important for the initial process of protein synthesis and for maintaining a redox environment	<i>Rv2400c, Rv2399c, Rv2398c, Rv2397c, Rv1286, Rv1285, Rv2131c, Rv2837c, Rv2392, Rv2391, Rv0331, Rv0815c, Rv2291, Rv3117, Rv3283, Rv2335, Rv2334, Rv3684, Rv3341, Rv1079, Rv0391 and Rv3238c</i>	C00033, C00042, C00053, C00054, C00059, C00065, C00084, C00087, C00094, C00097, C00155, C00224, C00245, C00263, C00283, C00320, C00409, C00580, C00979, C01118, C01861, C02084, C03920, C04022, C08276, C11142, C11143, C11145, C15521, C17267, C19692, C20870 and C20955	Mtu00920
Biotin/ Metabolism of cofactors and vitamins/ Necessary for lipid biosynthesis and gluconeogenes is	<i>Rv1350, Rv0242c, Rv3559c, Rv0769, Rv0032, Rv1569, Rv1568, Rv1570, Rv1589, Rv3279c and Rv1442</i>	C01209, C01894, C01909, C02656, C05552, C05921, C06250, C19673, C19845, C19846, C20372, C20373, C20374, C20375, C20376, C20377, C20378, C20384, C20385, C20386, C20387, C20683 and C22458	Mtu00780
Colesterol/ Lipid Synthesis of virulence- related lipids. Providing latent infection survival products	<i>Rv0764c</i>	C00187, C00448, C00751, C01054, C01164, C01189, C01561, C01673, C01694, C01724, C01753, C01789, C01802, C01902, C01943, C02141, C02530, C03428, C03845, C04525, C05103, C05107, C05108, C05109, C05437, C05439, C05440, C05441, C05442, C05443, C07712, C08813, C08821, C08830, C11455, C11508, C11522, C11523, C15776, C15777, C15780, C15781, C15782, C15783, C15808, C15816, C15915, C18231, C21106, C22112, C22116, C22119, C22120, C22121,	Mtu00100

C22122, C22123 and  
C22136.

The electron transport chain is formed for different complex of protein, and it is interesting to note that the MTB is extremely adapted to survive in different conditions of availability of energy substrates and oxygen concentration. The MTB can control your metabolism and utilized different sources of nutrients depends on your environment. In the case of aerobic conditions, like in exponential phase in vitro, the MTB uses a classical electron transport chain pathway. The classical electron transporter chain of MTB is formed by the complex Cox, Nuo, Sdh1, Hyd1, Qcr, Cta and participating of this pathway alanine dehydrogenase, proline dehydrogenase, glycerol3-phosphate dehydrogenase and Hyd2. In energy-limit conditions the complex Cox, Nuo, Sdh1, Hyd1 and Hyd2 are upregulated [50,129,132–134].

In case of limited concentration of oxygen, MTB uses an alternative electron transporter chain to made oxidative phosphorylation. This response for limitation in oxygen availability utilized alternate donors and acceptors of electrons, in this electron transporter chain the complexes are Mqo, Ndh, Sdh2, Hyd1, Nar, Cyd, Frd, Hyd3 and Hyd2. In this alternative electron transporter chain, the complexes upregulated are Mqo, Ndh, Sdh2, Hyd1, Hyd2, Hyd3, Cyd and Frd, Figure 4 [133,135].



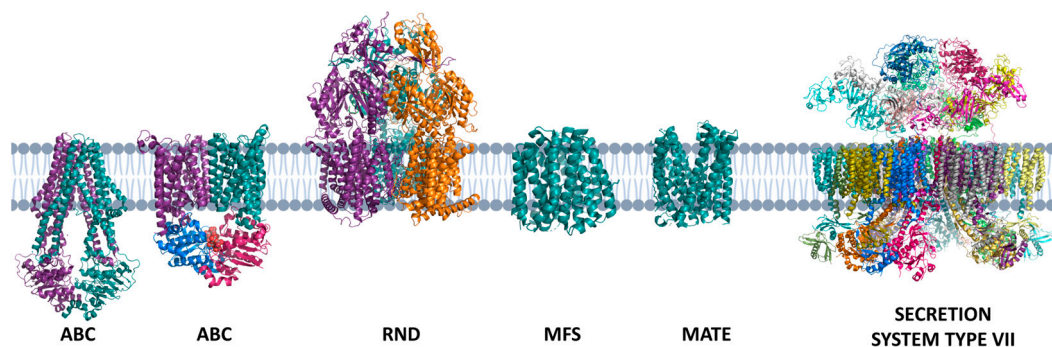
**Figure 4.** Comparison of electron transport chains. Point A represents the classic electron transport chain used in aerobic conditions, for example, in the in vitro phase, formed by the complex Cox, Nuo, Sdh1, Hyd1, Qcr, Cta participates in this pathway alanine dehydrogenase, proline dehydrogenase, glycerol3-phosphate dehydrogenase and Hyd2. Under energy boundary conditions, the Cox complex, Nuo, Sdh1, Hyd1 and Hyd2 are upregulated. Point B represents the alternative electron transport chain, used in case of limited oxygen concentration, to carry out oxidative phosphorylation, in which it makes use of alternative electron donors and acceptors, the complexes that make up the chain are Mqo, Ndh, Sdh2, Hyd1, Nar, Cyd, Frd, Hyd3 and Hyd2, and the upregulated complexes are Mqo, Ndh, Sdh2, Hyd1, Hyd2, Hyd3, Cyd and Frd.

## 5. Transport Substrates Across the Membrane: The Set Transporters from MTB

The MTB presents membrane proteins important for the transport of several substrates, these transport systems correspond to the four main superfamilies: ATP Binding Cassette (ABC) that are

considered primary transporters, since they use ATP as energy source for the system; Small Multidrug Resistance (SMR), Resistance Nodulation Division (RND), Major Facilitator Super-family (MFS), which are considered secondary transporters because they use the proton motor force ( $H^+$  or  $Na^+$ ) as energy. The Multidrug and Toxic-compound Extrusion (MATE) family is another important superfamily of transporters but not reported in MTB [103,136–141].

MTB has the ability to capture various carbon and nitrogen sources, organic and inorganic sources of phosphate and sulfur, many of which are transported by ABC transporters. In addition, probable MTB has the presence of porins in the mycomembrane without which the uptake and essential nutrients would not be possible. However, in MTB many of these transporters still need to be characterized. About 2.5% of the MTB genome encodes for components of importers ABC transporters with at least 37 complete transporters. Importers of carbohydrates such as the UgpABCE and SugABC; anions such as the PhoT, PstACBS, CysATW and ModABC; and peptides, DppABCD and OppABCD. In the case of exporters, MTB has exporters of polysaccharides, lipids, virulence factors and antimicrobial peptides (AMPs) [142–144].



**Figure 5.** MTB transporters. MTB presents different types of transporters that act as importers and exporters. The main MTB transporters are ABC, RND, MATE and MFS type. Also noteworthy is the presence of 5 type VII secretion systems that act on MTB virulence.

Most important are multidrug exporters ABC transporters that cause resistance to antibiotics used in the treatment of tuberculosis. Currently, four drugs are used: isoniazid (INH), pyrazinamide (PZR), ethambutol (ETH) and rifampicin (RIF) during the first two months, and the combination of rifampicin and isoniazid for another four months. The increased incidence and spread of MDR (Multi-Drug Resistance) and extensive drug resistance (XDR) has become a serious public health problem. MDR strains are resistant to isoniazid and rifampicin, first-line drugs, and XDR strains are resistant to these and to fluoroquinolones and injectable aminoglycosides [140,145–147].

Some ABC transporters already have a direct relationship with resistance, such as Rv1217c/1218c (RIF and INH), Rv2686c/2687c/2688c [fluoroquinolones (FLQ), such as ciprofloxacin (CPX)], and Rv1456c/1457c/1458c [RIF, INH, ETH and streptomycin (STM)]. The drug exporter best characterized in MTB and responsible for a significant increase in resistance is the DrrA/DrrB, where DrrA is the ATPase domain and the DrrB the transmembrane domain. The expression of this drug caused an increase in MIC (Minimum Inhibitory Concentration) for daunorubicin (DRB), doxorubicin (DOX), puromycin (PUR), norfloxacin (NOR), chloramphenicol (CLOR), tetracycline (TET), anthracyclines (ANT), and ETH and STM. Finally, the Rv0194 transporter, consisting of a single gene coding for both transmembrane domains and ATPase, is a multidrug efflux pump, which promotes the increase of MIC for ampicillin (AMP), vancomycin (VAN), erythromycin (ERY), novobiocin (NOV), STM, TET and CLOR [148–151].

In addition, metals like copper need to be exported out or metabolized by MTB because are toxic for the bacteria. The MTB encodes a copper transporter, in addition to a copper-binding metallothionein, which demonstrate the presence of a detoxifying metabolism for that metal [152].

The MFS transporters are a large family of transporters that includes uniporters, symporters and antiporters. MFS has 12 or 14 transmembrane helix and transport many types of substrates, like



anions, cations, amino acids, simple sugars, and drugs. The MTB has 16 open reading frames identified as MSF type efflux pumps; the Rv1634 as associated to fluoroquinolone resistance to NOR and CPX; Rv1258 confer resistance to TET, and aminoglycosides like STM; and EfpA increase the resistance to INH and other antimicrobial compounds [153].

SMR transporters are formed by homodimers or heterodimers and have four transmembranes. The protomers are antiparallel, three transmembranes are responsible for forming the pore, and the fourth transmembrane is responsible for dimerization. Only Rv3065 was described in MTB and is responsible for erythromycin (ERY), acriflavine and ethidium bromide [154,155].

MTB also has members of the RND family; these transporters are presented as homotrimers with 12 transmembranes and a large periplasmic region. The MTB has 15 putative RND transporters known as MmpI (mycobacterial membrane protein, large), these proteins are apparently similar in structure and sequence. Four of these proteins has in our operons mmpS (mycobacterial membrane protein, small), and would have a role of adapters for the formation of a channel to the micromembrane. MmpL proteins are linked to the transport of lipids, whereas MmpL6 is part of a detoxification system of INH and other compounds. The bacillus also presents mechanosensitive channels that are activated by the increase of tension in the bilipid layer. These channels during the severe osmotic downshock can create large pores in the membrane in a transient way that collaborate for the relief of the tension and guarantee the maintenance of cellular integrity [75,79,156–160].

The Type VII secretion system (T7SS), also known as ESX (ESAT-6 secretion) system, is a specialized protein secretion system found in mycobacteria and other Actinobacteria. It is involved in the secretion of effector proteins across the bacterial cell envelope, playing crucial roles in virulence, pathogenesis, and bacterial survival. The T7SS is composed of a complex machinery of proteins that work together to facilitate the secretion process. The core components of the T7SS include membrane-associated ATPases (EccB and EccC), membrane proteins (EccD and EccE), and a substrate recognition complex (EccB1-EccC1) [3]. These components form a transmembrane channel that spans the mycobacterial cell envelope and allows for the secretion of effector proteins [161–163].

The T7SS is classified into five paralogous loci, known as *esx-1* to *esx-5*, which are present in different combinations in various mycobacterial strains. Each locus encodes a distinct set of proteins that contribute to the overall function of the T7SS. For example, the *esx-1* locus is essential for the virulence of *Mycobacterium tuberculosis*, while the *esx-3* locus is involved in iron acquisition and nutrient uptake. The secretion of effector proteins through the T7SS is mediated by specific secretion signals present in the substrates. These signals include conserved motifs such as YXXXD/e and WXG, which are recognized by the substrate recognition complex and facilitate the translocation of the effector proteins across the cell envelope [164–168].

The T7SS has been implicated in various functions that contribute to bacterial fitness and survival. It is involved in the modulation of host immune responses, allowing mycobacteria to evade the immune system and establish persistent infections. The T7SS also plays a role in nutrient acquisition, including iron uptake, which is crucial for mycobacterial growth and survival. Additionally, the T7SS has been implicated in interbacterial competition, where it can secrete toxins that target competitor bacteria, providing a selective advantage for the secreting strain [161,162,167–169].

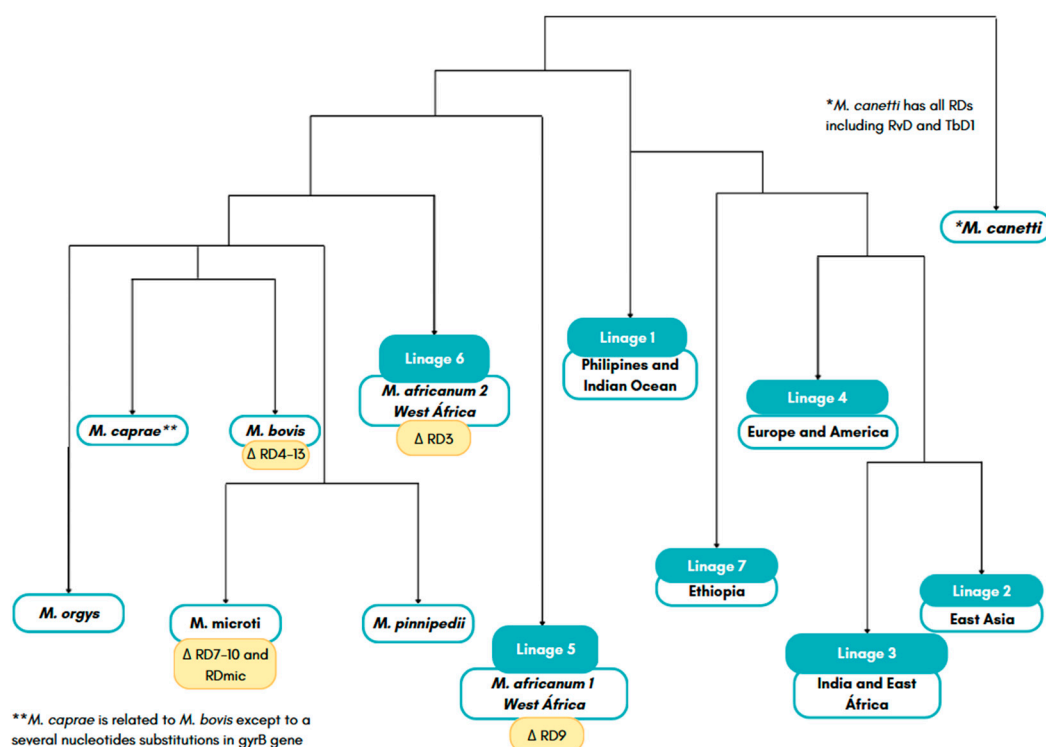
## 6. Who Are MTB? The Genes Answer the Question

The MTB lineage H37Rv (most common and first to be fully sequenced) has a genome consisting of approximately 4000 genes, 225 genes linked to lipid metabolism, 227 information pathways, 517 cell wall and cellular processes, 50 stable RNAs which include rRNA and tRNA, 137 insertion elements and bacteriophages, 167 of PE and PPE proteins that are acidic and rich in N-terminal proline and glutamate, 877 genes concerning intermediate and respiration metabolism, 190 regulatory protein genes, 91 genes linked to virulence, detoxification and adaptation, 911 hypothetical conserved function genes and 605 genes of unknown function [170,171].

About 190 proteins encoded by the MTB genome are regulatory proteins, of which 11 correspond to two-component systems, five unpaired response regulators, two unpaired kinases, 11 protein

kinases and another 140 regulatory transcripts. They are involved in situations of hypoxia, thermal shock, lack of nutrients, lack of metals, and responses to oxidative stress and reactive nitrogen species. MTB as well as other bacteria exhibits DNA replication from an OriC origin to the terminal *ter* region, in a process that occurs in two directions of the circular genome. The ribosomes have the same fractions seen in *E. coli*: 16s, 23s and 5s and MTB rRNAs are encoded by the *rrn* operon, which in MTB corresponds to a single operon [172–174].

MTB complex can be divide in human-adapted pathogens, which 5 lineages (lineage 1: the Philippines and Indian Ocean; lineage 2: East Asia; lineage 3: India and East Africa; lineage 4: Europe and Americas; lineage 7: Ethiopia), the *Mycobacterium africanum* with 2 lineages (lineage 5: *M. africanum* 1 in West Africa; lineage 6: *M. africanum* 2 in West Africa) and the animal-related species like *M. bovis*, *M. pinnipedii*, *M. microti* and *M. orygis*; and the *M. canetti* in East Africa. Except by *M. canetti*, the members of MTB complex have high genomic similarity, and the differences are found in single nucleotides polymorphisms (SNPs) and deletions in regions called regions of difference (RDs), besides phenotypic differences in virulence in host adaptability, Figure 6 [175–179].



**Figure 6.** MTB lineages adapted to man. Lineage 1: Philippines and Indian Ocean; Lineage 2: East Asia; lineage 3: India and East Africa; Lineage 4: Europe and the Americas; Lineage 5: *M. africanum* 1 in West Africa; Lineage 6 *M. africanum* 2 in West Africa; Lineage 7: Ethiopia; And species related to animals such as: *M. bovis*, *M. pinnipedii*, *M. microti*, *M. orygis*; and *M. canetti*. Also, which deletions from RDs 1 to 14 do some strains have; strain 5 RD9, strain 6 with RD3, *M. microti* with RD7, RD8, RD9, RD10 and RDmic and *M. bovis* with RD4, RD5, RD6, RD7, RD8, RD9 and RD10; And *M. canetti* has no deletion, harboring all RDs including RvD and TBD1.

The studies help to characterize 14 RDs (RD1-14) presents in *M. tuberculosis* H37Rv, while the regions (RvD1-5) are absent in the strain H37Rv in addition to a specific deletion region known as TbD1. *M. canetti* has all RDs including RvD and TbD1, while *M. africanum* from West Africa lacks RD9 and the isolates from East Africa lacks RD3. The *M. microti* lacks RD7, RD8, RD9 and RD10, besides a specific region called RDMIC, *M. bovis* shows deletions of RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD12 and RD13; while The BCG strain show deletions of the RD1, RD2 and RD14 that occurs in the attenuation process. Finally, the *M. caprae* is related to *M. bovis* except to a several nucleotides substitutions in *gyrB* gene. For example, the RD1 region in MTB is 9.1 kilobases in size, it consists of

9 genes, among them the gene coding for the protein 10-kDa CFP-10 and ESAT-6. The genes surrounding these two proteins encode specialized secretion systems. In addition, MTB has four more locus of secretion homologous to that found in the RD1 region [179–182].

The MTB presents two families of genes that are unique in the genus *Mycobacterium*, families PE and PPE that encode proteins very similar to ESAT-6 and CFP-10 proteins. In the pathogenic mycobacteria, these two families of genes are present in great quantity when compared with other gene families [183–185].

In *Mycobacterium tuberculosis*, several types of non-coding RNA have been identified, including antisense transcripts, riboswitches, and small intergenic RNAs (sRNAs). Antisense transcripts are short RNA molecules that base-pair with the 5' regions of mRNAs. They have been found to regulate gene expression in *M. tuberculosis*, including genes involved in the transposition of foreign genetic elements. These antisense transcripts can range in size from less than 50 nucleotides to several kilobases and can map to sequences throughout the length of cognate mRNAs [186,187].

Riboswitches are RNA elements that can bind specific ligands and regulate gene expression. In *M. tuberculosis*, riboswitches have been identified, such as the cobalamin riboswitch, which represses gene expression in the presence of specific ligands. Small intergenic RNAs (sRNAs) are short transcripts that are encoded between open reading frames. They can regulate gene expression by base-pairing with target mRNAs, thereby modulating translation efficiency and mRNA stability. These sRNAs have been shown to be differentially expressed in response to changing environments and may play important roles in the regulation of gene expression and growth in *M. tuberculosis* [188,189].

The presence of these non-coding RNAs in *M. tuberculosis* suggests that post-transcriptional regulation plays a significant role in the pathogenesis of the disease. They have the potential to regulate the expression of genes involved in virulence and adaptation, contributing to the ability of the bacterium to cause disease and persist in the host [186,187].

A regulatory component of MTB gene transcription consists of the *DosR-DosS/DosT* system, which controls other regulons known to activate the bacillus dormancy stages under microaerophilic conditions. This regulon also controls genes linked to the production and activation of respiration using alternative electron acceptors. Two sensors that are histidine kinases proteins, DosS and DosT, phosphorylate the Dos regulon. Once activated this regulon controls about fifty genes directly, many of which still have unknown function. It is known that besides the hypoxia condition, situations such as nitric oxide concentration also activate this regulon. The redox stress that is inside the macrophage is also another factor that can activate the Dos regulon. Although this regulon is activated in the early stages of hypoxia, studies have shown that it is not essential for the survival of bacillus in the state of dormancy. It is also noted that in mutants with deletion in genes coding for heat shock proteins, the ability to remain inside macrophages is lost [135,190–195].

MTB presents three distinct systems for DNA repair, such as homologous recombination, non-homologous junction and single-strand annealing. Each of these repair systems is activated according to the type of lesion that has occurred and its extension, in addition, are extremely coordinated processes by different sets of genes and proteins. MDR strains have shown mutations in genes linked to DNA repair, which may be the cause of hypermutations in the genes that code for the targets of the drugs and that, therefore, would promote a rapid adaptation of the strain to the treatment and action of the immune system [196–202].

In MTB the genes that are regulated by the *sigH*, *rpoV* and *whiB3* genes are related to controls of host immune response that facilitate the spread of MTB to new individuals. Furthermore, MTB have a set of genes designated to lipid and fatty acid metabolism, including the catabolism of cholesterol; inhibitors of antimicrobial effectors of macrophage, like inhibitors of apoptosis, proteins to oxidative and nitrosative stress and phagosome arresting; metal transporters, importers and exporters; proteases, including metalloproteases and  $\beta$ -lactamases; cell wall proteins, like lipoproteins and secretion systems; gene expression regulators, including sigma factors, two component systems and transcriptional regulators; and others destined to survive and virulence of MTB [203–206].

MTB presents 13 sigma factors currently described, all belonging to the  $\sigma 70$  family, which recognizes regions -35 and -10 of the promoter. Interestingly, MTB does not show any sigma factor of the  $\sigma 54$  family, which recognizes regions -12 and -24 of the promoter. The  $\sigma 70$  MTB, present representatives of the 4 groups of this family: Group 1, represented by  $\sigma A$ ; group 2, represented by  $\sigma B$ ; group 3, represented by  $\sigma F$ ; and finally, another 10 sigmas representing group 4. The factors  $\sigma A$  and  $\sigma B$  are present in all mycobacteria, as well as  $\sigma E$ , the sigmas  $\sigma D$ ,  $\sigma F$ ,  $\sigma G$ ,  $\sigma H$  and  $\sigma J$  are found in all mycobacteria except *M. leprae*. However,  $\sigma C$  is present only in pathogenic mycobacteria, Table 4 [207–214].

Another regulation in gene transcription can be performed through anti-sigmas. These proteins interact with sigma factors, preventing them from binding to the promoter or promoting the disengagement of the factor from promoter. In MTB the anti-sigmas RsmA,  $\sigma M$  anti-sigma were described; RseA,  $\sigma E$  anti-sigma; RshA,  $\sigma H$  anti-sigma; RslA,  $\sigma L$  anti-sigma; RskA,  $\sigma K$  anti-sigma; RsdA,  $\sigma D$  anti-sigma; RsfA,  $\sigma F$  anti-sigma. Anti- sigma Rv0093c and Rv0181c, which are sigma anti-sigma  $\sigma C$  and  $\sigma G$ , have been identified putatively. The anti-sigmas  $\sigma E$ ,  $\sigma F$  e  $\sigma H$  were identified as proteins present in the cytoplasm, whereas the other anti-sigmas would be proteins located in the plasma membrane.

In addition, anti-anti-sigma factors, whose function is to block anti-sigmas, are Rv1635c, Rv3687c, Rv0516c, Rv1364c, Rv1904 and Rv2638 blockers of  $\sigma F$  anti- sigma. Interestingly, some sigma factors self-regulate their levels, binding in their own promoters, is the case of factors  $\sigma A$ ,  $\sigma B$ ,  $\sigma D$ ,  $\sigma F$ ,  $\sigma E$ ,  $\sigma H$ ,  $\sigma K$ ,  $\sigma L$  and  $\sigma M$ . While sigmas also regulate other sigmas, as is the case of  $\sigma H$  regulates the sigmas  $\sigma B$  and  $\sigma E$ ; the  $\sigma E$ , regulates the  $\sigma B$ ; which is also regulated by sigma  $\sigma L$  and  $\sigma F$ . The  $\sigma F$  also regulates the  $\sigma C$ . The  $\sigma G$  also regulates the sigmas  $\sigma H$  and  $\sigma F$ . Finally,  $\sigma J$  regulates the factor  $\sigma I$ . Regarding the MTB promoters, research indicates that the -10 region of the promoters are similarity to the *E. coli* promoters, however, the -35 region appears to be totally different from the regions described for *E. coli*. In addition, there is variation in the distance between these two regions when compared to these two bacteria, as in *E. coli* an AT-rich region at position -15 is observed, due to the high content of CG present in the genome, is absent in MTB[154,204,212–216].

**Table 4.** Sigma factor of MTB.

Sigma	Presence	Response	Observation
$\sigma^A$	All mycobacteria	Nutritional deficiency and the general response to the stress	During the infection it was observed that its expression increases in some strains but not for the H37Rv strain
$\sigma^B$	All mycobacteria	Nutritional deficiency and the general response to the stress	It is not essential for the survival of the bacillus
$\sigma^C$	All pathogenic mycobacteria	All pathogenic mycobacteria	Act on the Immunopathological phenotype of tuberculosis
$\sigma^D$	All mycobacteria except <i>M. leprae</i>	It regulates genes linked to starving response during nutritional deficiency	Act on the Immunopathological phenotype of tuberculosis



$\sigma^E$	All mycobacteria	Action during stress on the bacillary surface and during the response to thermal shock	Influence on the immunopathological phenotype
$\sigma^F$	All mycobacteria except <i>M. leprae</i>	Involved in the biosynthesis of the mycobacterial envelope	Influencing the immunopathological phenotype
$\sigma^G$	All mycobacteria except <i>M. leprae</i>	Acts on the SOS response, a global response to DNA damage, and acts on the survival of the bacillus during macrophage infection	Present as characteristics the presence of over 120 amino acid residues in the C-terminal
$\sigma^H$	All mycobacteria except <i>M. leprae</i>	Includes participation during oxidative stress, heat stress	Influence on the immunopathogenic phenotype
$\sigma^I$	Only in pathogenic mycobacteria	It is induced during thermal shock processes	Present as characteristics the presence of over 120 amino acid residues in the C-terminal
$\sigma^J$	MTB	Acting during oxidative stress	Present as characteristics the presence of over 120 amino acid residues in the C-terminal
$\sigma^L$	MTB	Is bound to the virulence and biosynthesis of phthiocerol dimycocerosate	An essential lipid for the virulence of MTB
$\sigma^M$	MTB	Is responsible for the long-term adaptation of the bacillus in vivo	-
$\sigma^K$	MTB	Yet its performance is unknown	-

In MTB the virulence and pathogenesis are directly linked to its ability to survive within the host. The disease is a consequence of the survival and replication of MTB in the host and its relation to the immune system. It is interesting to note that MTB is extremely adapted to survive under the extreme conditions that lie within the macrophages and other cells of the immune system. In addition to possessing an incredible capacity to modulate not only its metabolism and cell division under these conditions but also, manipulate the immune responses of the host [16,118,180,217–220].

A curious fact of MTB is the absence of classical virulence factors such as toxins. However, the presence of several genes whose functions allow the survival, adaptation, persistence, control and dissemination of MTB in the host are observed. The main virulence factors of MTB are discussed below, Table 5 [128,221–235].

**Table 4.** Sigma factor of MTB.

Gene	Function	Category
<i>Rv2940</i>	Promotes the synthesis of long chain fatty acids	Cell Wall
<i>Rv2930</i>	Acyl-coenzyme A, promotes the degradation of fatty acids	
<i>Rv2941</i>	Fatty-acid-CoA synthetase that acts in the lipidic pathway	
<i>Rv2942</i>	Promotes the transport of lipids and synthesis of the mycomembrane	
<i>Rv3804c</i>	Promotes the transfer of mycolic acids to trehalose	
<i>Rv0642c and Rv0410</i>	A methyltransferase bound to the synthesis of mycolic acids	Cell Wall
<i>Rv0899</i>	It is a protein expressed at low pH	
<i>Rv0475</i>	It is a heparin-binding hemagglutinin protein	
<i>RD1 region</i>	Inhibition of phagosome maturation and apoptosis. Responsible for encoding a network of secretion systems	
<i>Rv2246</i>	It is a culture filtrate protein whose function is a chaperonin linked to latency and persistence	
<i>Rv3763</i>	It is a culture filtration protein that promotes the regulation of IL-12	Cell Wall
<i>Rv1811</i>	Acts on magnesium uptake	
<i>Rv3083 to Rv3089, and Rv2869c</i>	MTB related to mycolic acid synthesis is <i>mymA</i>	

operon		
<i>Rv2946C, Rv1660, Rv2048c, Rv2941, Rv2938, 1527c, Rv1661, Rv3823c, Rv1345 and Rv1916</i>	Linked to complex lipid synthesis	
<i>Rv1411c, Rv1410c, Rv0934, Rv1235 and Rv1857</i>	Lipoproteins that constitute virulence factors	
<i>Rv3682</i>	Transglucosylases and transpeptidase	
<i>Rv2136</i>	Involved in the synthesis of peptidoglycan	
<i>Rv0198c and Rv2869c</i>	Metallo-proteases	
<i>Rv2097c and Rv2115c</i>	Proteasome associated proteins	
<i>Rv2382c, Rv1348, Rv1349, Rv2711 and Rv1811</i>	Related to metal transporter	
<i>Rv3270</i>	Zinc efflux	
<i>Rv0969</i>	Copper efflux	
<i>Rv3367, Rv1818c and Rv2136c</i>	Rv3367, Rv1818c and Rv2136c	Cell Wall
<i>MT18B_4990, Rv1411c, Rv1270c and Rv0934</i>	Lipoproteins that are Toll Like Receptor-2 (TLR2) agonists and their Myeloid Differentiation Primary-Response protein 88 (MYD88)	
<i>Rv0350, Rv1860, MT18B_4990 and Rv1436</i>	Allow the bacillus to bind to cells, either phagocytosed and continue to replicate	Intermediary metabolism and respiration
<i>Rv2220</i>	A glutamine synthetase, also constitutes a culture filtration protein, acts on the metabolism of	

nitrogen		
<i>Rv0467</i>	Isocyttrase lyase, converts isocitrate to succinate and allows bacterial growth under fatty acids and acetate	
<i>Rv3487</i>	It is a lipase esterase that acts on lipid degradation	
<i>Rv1345</i>	Acts on $\beta$ - oxidation of fatty acids	
<i>Rv2351c, Rv2350c, Rv2349c and Rv1755c</i>	Phospholipases involved in the cycles of obtaining energy	
<i>Rv3602c and Rv3601</i>	Consist of pantothenate synthase proteins, this molecule that acts on the degradation of lipids and other cell signaling	
<i>Rv2987, Rv2192, Rv0500 and Rv0780</i>	Act on the biosynthesis of leucine, tryptophan, proline and purines respectively	
<i>Rv1161</i>	Involved with respiration under anaerobic conditions and the conversion of nitrate to nitrite	
<i>Rv0475, Rv0930, Rv0820, Rv2224c, Rv3236c, (Rv3666c to Rv3663c) and Rv2200c</i>	Linked to cell wall	
<i>Rv3883c</i>	Proteases involved with virulence	Intermediary metabolism and respiration
<i>Rv0983 and Rv3671c</i>	Serine proteases	
<i>Rv3810</i>	Act with multiplication and intracellular growth	
<i>Rv3671c</i>	Encodes a membrane protein responsible for MTB resistance to the acidic environment of IFN- $\gamma$ -activated phagosomes	



<i>Rv0195, Rv0386, Rv0491, Rv0890c, Rv0894, Rv3416, Rv3133c, Rv1013, Rv2946c, Rv2488c and Rv3133c</i>	Formation of biofilms. This structure is involved in bacterial persistence and protects it from chemical and physical agents	
<i>Rv2711</i>	Binds to regions of genes involved in iron uptake and nitrate reductase	
<i>Rv0757</i>	Controls the expression of virulence genes by magnesium deficiency	Regulator Proteins
<i>Rv0903c</i>	Regulates macrophage virulence genes	
<i>Rv0981</i>	Two-component system that regulates macrophage virulence genes	
<i>Rv3416</i>	Cytoplasmic redox sensor, linked to pH resistance	
<i>Rv0931c and Rv0410c</i>	Proteins kinases related to virulence	Regulator Proteins
<i>Rv2745c</i>	ATP-dependent protease	
<i>Rv1908c</i>	Catalase: peroxidase that degrades peroxides and other organic peroxides	
<i>Rv2428</i>	Protein whose function is to detoxify hydroperoxides	
<i>Rv3846 and Rv0342</i>	Act in the detoxification of superoxide	Virulence, detoxification, adaptation
<i>Rv0432</i>	Superoxide dismutase	
<i>Rv1936 to Rv1941 and Rv1908c</i>	Catalase-peroxidase protein	
<i>Rv1932</i>	Thiol peroxidase	

<i>Rv0353</i>	gene repressor of proteins of heat shock	
<i>Rv0251c</i>	Possibly a molecular chaperone	
<i>Rv3409c, Rv3568, Rv34545c, Rv3544c, Rv3543c, Rv3542c, Rv3541c and Rv3540c</i>	Linked to catabolism of cholesterol	
<i>Rv2383c</i>	Linked to production mycobactin, an important siderophore in MTB	Lipid metabolism
<i>Rv2246</i>	protein involved in lipid and fatty acid metabolism	
<i>Rv3151, Rv1743, Rv3654c and Rv3655c</i>	Involved with the inhibition of apoptosis	Conserved hypotheticals
<i>Rv2027c, Rv0490, Rv0981, Rv0982, Rv2395A and Rv2395B</i>	Regulatory proteins	
<i>Rv2032, Rv0211, Rv0153c and Rv0990c</i>	Virulence factors are found the region of difference RD2	Conserved hypotheticals
<i>Rv2445c, Rv2234 and Rv1651c</i>	Involved in phagosome arresting	PE/PPE

## 5. Conclusions

The ability of MTB to survive within the macrophage and to spread during active disease is well known. MTB is one of the most adapted bacteria to survive against the human immune system and demonstrates great metabolic and regulatory versatility that allowed it to evolve with its host for thousands of years.

The MTB cell wall is extremely complex and protects against various external agents. There is still much debate about its organization; could mycolic acids be organized in a similar way to the Gram-negative outer membrane? More studies are needed to better understand their characteristics and components.

The pathogenic capacity of the MTB is due mainly to its unique characteristics, whether genetic, proteomic or metabolic. Understanding the biology of these microorganisms is extremely important, as it is one of the world's major infectious diseases with few treatment options.

In addition, a better understanding of the physiology and molecular biology of MTB will allow the development of new diagnostic and treatment techniques, which are currently scarce due to the emergence of resistant strains.

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