

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The Cord Factor: Structure, Biosynthesis and Application in Drug Research – Achilles Heel of *Mycobacterium tuberculosis*?

Ayssar A. Elamin, Matthias Stehr and Mahavir Singh

Department of Gene Regulation and Differentiation,
Helmholtz Centre for Infection Research, Braunschweig
Germany

1. Introduction

Recently, it was reported that the tuberculosis mortality in 2009 has dropped to 35% since 1990 (WHO, 2010). Nevertheless, the disease caused by the facultative intracellular bacterial pathogen *Mycobacterium tuberculosis* still remains the leading cause of death from a single bacterial species (Coker, 2004; Russell et al., 2010). The emergence of Multi-Drug Resistant (MDR) and Extreme Drug Resistant (XDR) strains of *M. tuberculosis* leads to prolonged treatment which drastically increases the therapy costs.

M. tuberculosis shows a remarkable property of existing in different states of invasion (infection), colonization and persistence (Casadevall & Pirofski, 2000). It also has outstanding mechanisms to escape from elimination and has a high degree of intrinsic resistance to most antibiotics, chemotherapeutic agents and immune eradication (Brennan & Nikaido, 1995; Coker, 2004). The major obstacle for host defence mechanisms and therapeutic intervention is the unusual robust cell wall which is unique among prokaryotes, and is a major determinant of virulence of the bacterium. The cell wall is critical for long-term persistence of *M. tuberculosis* in the hostile environment of the host's cells and for progression of tuberculosis (Barry et al., 1998). Approximately one-half of the cell wall mass is comprised of mycolic acids (Brennan & Nikaido, 1995). In the cell envelope, mycolic acids are esterified to the terminal pentaarabinofuranosyl unit of arabinogalactan, which is a peptidoglycan-linked polysaccharide. The outer envelope consists of trehalose 6,6'-dimycolate (TDM; cord factor) and TMM (trehalose 6,6'-monomycolate, the biosynthetic precursor of TDM), where the mycolic acids of TDM interact with the mycolyl-residues from the layer beneath (Brennan & Nikaido, 1995). The mycolic acid-containing layers have width of ~10 nm and limit the penetration of hydrophilic substances, whereas the inner saccharide layer inhibits the penetration of lipophilic substances. The high abundance of mycolic acids in the outer cell envelope is the main barrier for water soluble antibiotics (Brennan, 2003; Coker, 2004).

The purpose of this review is to highlight the importance of the cord factor as one of the most unique determinant for *Mycobacterium tuberculosis* virulence. This article will especially focus on the steps of the cord factor biosynthesis, i.e., the transfer of mycolic acid from a

TMM to another TMM to form TDM by Ag85 complex enzymes. The Ag85 complex is one of the promising targets for novel antimycobacterial drugs and vaccines. We present our recently developed high throughput screening (HTS) assays suitable for the identification of potential inhibitors against Ag85.

2. Discovery of the cord factor (Trehalose 6,6'-di-mycolate; TDM)

In 1884, Robert Koch described *M. tuberculosis* bacilli grown in culture as rope-like structures (Koch, 1884). More than half a century later, in 1947 Middlebrook found that the ability to form cords under specific conditions is an “essential accompaniment of virulence” (Middlebrook et al., 1947). In 1950 Bloch extracted the substance responsible for cord formation from virulent organisms and identified it as a “toxic substance” (Bloch, 1950).

The removal of the substance with petroleum ether resulted in somehow avirulent organisms but did not affect the growth of the bacilli. This suggested that the substance was located at the surface and since it was obtained only from “cordforming” organisms it was called “cord factor” (Bloch, 1950; Middlebrook et al., 1947). Six years later the cord factor was finally identified as trehalose 6,6'-di-mycolate (TDM) by Noll (Behling et al., 1993a; Noll, 1956). TDM is the most abundant glycolipid produced by virulent *M. tuberculosis* (Hunter et al., 2006a). TDM molecules consist of trehalose (TDM glycan-head). Trehalose is abundant in mycobacteria as a free component (Elbein & Mitchell, 1973; Elbein et al., 2003). In the cord factor trehalose is esterified to two mycolic acid residues and the residues length is variable from species to species (Fig. 1). Mycobacterial mycolic acids contain generally 20–80 carbons (Spargo et al., 1991).

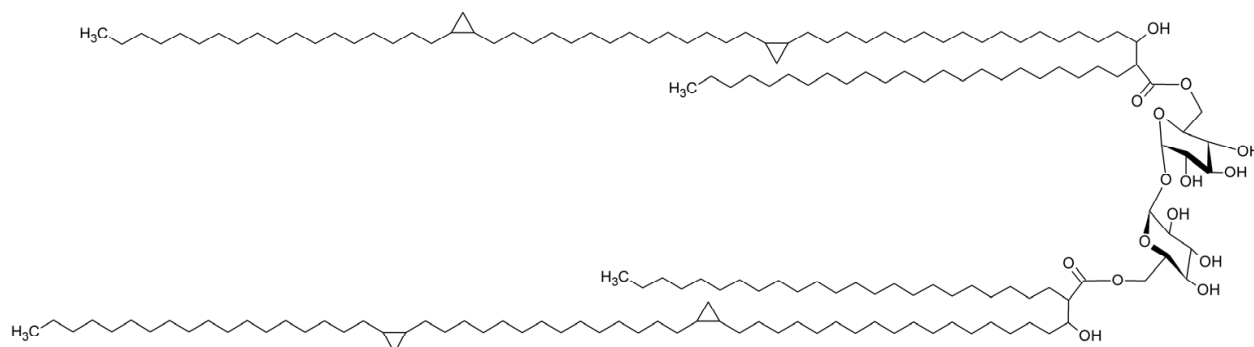


Fig. 1. Structure of trehalose 6,6'-di-mycolate (TDM, cord factor).

The cord factor is comprised of trehalose which esterified to two mycolic acid residues through their 6- and 6'-hydroxyl groups.

2.1 Effects of the cord factor on the immune system

TDM inhibits the process of phagosome-lysosome fusion and is thus a key compound for the survival of the bacillus inside the host's phagosomes (Indrigo et al., 2002). TDM induces a broad range of cytokine secretion in the host's immune system, especially production of IL-1 β , IL-6, and TNF in macrophages (Matsunaga & Moody, 2009). In the recent years it has been shown that TDM is a key driver of secondary and cavitary disease type of tuberculosis (Hunter et al., 2006b). Despite the various severe effects on the host immune system the host

receptor could not be identified. In 2009 Ishikawa et al. could demonstrate that macrophage inducible C-type lectin (Mincle) is an essential receptor for TDM (Ishikawa et al., 2009).

2.2 TDM as vaccine adjuvant

In the last years TDM has been used intensively as immunomodulatory and vaccine adjuvant (Behling et al., 1993b; Noll, 1956). TDM can reproduce several pathophysiologic properties of *M. tuberculosis* infection including granuloma formation and induction of proinflammatory cytokines, such as IL-1 β , IL-6, and TNF in macrophages (Matsunaga & Moody, 2009). TNF (cachexin) has several toxic effects on host physiology, including sepsis, fever syndromes and cachexia (Silva & Faccioli, 1988).

Doses as little as 1–5 μ g are granulomagenic in the lungs of mice (Bekierkunst et al., 1969). Moreover TDM increases the production of antibodies (Behling et al., 1993b; Perez et al., 1994; Perez et al., 2000) and it up-regulates the expression of MHCII on macrophages (Ryll et al., 2001). TDM also induces *in vivo* production of IL-12 (Oswald et al., 1997). Injected as mineral oil solution (Silva & Faccioli, 1988) TDM forms monolayers, where the mycolic acids are exposed. In the monolayer form TDM is highly toxic (lethal dose in mice: LD₅₀ ~30 μ g) and kills macrophages in minutes (Hunter et al., 2006b). In aqueous suspension TDM forms micelles, where the mycolic acid groups are completely covered and TDM is non-toxic (lethal dose in mice: LD₅₀ >50,000 μ g). Micellar TDM prevents phagosome/lysosome fusion and thus promotes the survival of mycobacteria in the macrophage. Nevertheless, until now there is no experimental evidence for the existence or formation of TDM micelles or monolayers *in vivo*. Due to its strong immunostimulatory effect, several studies have used TDM as a potential adjuvant in different vaccination models. In 1976 Saito et al. were the first who described the cord factor as good adjuvant in mice and rats but with only low adjuvant effect in guinea pigs (Saito et al., 1976). Lima et al. could show that microspheres, containing TDM with a Hsp65-encoding DNA plasmid, were able to protect vaccinated mice against virulent *M. tuberculosis* (Lima et al., 2003) and against *Leishmania major* infection (Coelho et al., 2006). The major problem using TDM as adjuvant is the relatively high toxicity of the mycolic acids and the accompanying contaminants during the preparation of TDM. A synthetic analog of the cord factor, trehalose-6,6-dibehenate (TDB), was shown to be an effective and safe alternative (Davidsen et al., 2005). TDB is less toxic compared with TDM and easier to produce, making it a potent candidate in the field of vaccine development.

3. Drug targets in the biosynthesis of the cord factor

The cord factor (trehalose 6,6'-di-mycolate) is composed of a sugar and a mycolic acid component. In the following section we present the trehalose and mycolic acid biosynthesis steps and the target enzymes in their biochemical context. Especially enzymes of mycolic acid biosynthesis, such as methyl transferase (PcaA) (Glickman et al., 2000), β -ketoacyl-acyl carrier protein synthase (KasAB and FabH) (Bhatt et al., 2007), acyl-AMP ligase (Fad32) (Portevin et al., 2005) and polyketide synthase (Psk13) (Portevin et al., 2004), are regarded as promising targets for anti-TB drug development.

3.1 Biosynthesis of trehalose

Mycobacteria possess three pathways for trehalose synthesis (Kaur et al., 2009). Trehalose can be synthesized from glucose-6-phosphate catalyzed by trehalose-6-phosphate synthase (OtsA, *Rv3490*) (Pan et al., 2002) and trehalose-6-phosphate phosphatase (OtsB2, *Rv3372*) (Pan et al., 2002). The second pathway generates trehalose from glycogen involving the maltooligosyltrehalose synthase (TreY, *Rv1653c*) and the maltooligosyltrehalose trehalohydrolase (TreZ, *Rv1562c*). In the third pathway maltose is converted to trehalose by the trehalose synthase (TreS, *Rv0126*). While all the three pathways are functional and essential for the proliferation of *M. smegmatis* (Woodruff et al., 2004), the OtsAB pathway is predominant and strictly essential in *M. tuberculosis* (Fig. 2). In the genome sequence of *M. tuberculosis* exist two *otsB* homologues, but only OtsB2 (*Rv3372*) has a functional role in the pathway. OtsB2 has been suggested as an attractive target for novel drugs due to absence of trehalose in mammalian cell (Murphy et al., 2005).

3.2 Trehalose transporters

The LpqY-SugA-SugB-SugCATP-binding cassette transporter is highly specific for uptake of the disaccharide trehalose. Since trehalose is not present in mammals, it is unlikely that this system is used for sugar acquisition from the host. Trehalose release is known to occur as a byproduct of the biosynthesis of the mycolic acid cell envelope by *M. tuberculosis* antigen 85 complex. The mycolyltransferases of the antigen 85 complex transfer the lipid moiety of the glycolipid trehalose monomycolate (TMM) to arabinogalactan or another molecule of TMM, yielding trehalose dimycolate. These reactions lead to a constant release of trehalose from the cell. The LpqY-SugA-SugB-SugC ATP-binding cassette has been suggested as transporter system (Fig. 2), recycling the released trehalose. Perturbations in trehalose recycling strongly impaired virulence of *M. tuberculosis*. (Kalscheuer et al., 2010). These sugar transporters are thought to play an important role in bacterial pathogenesis and have been suggested as target for tuberculosis chemotherapy (Kalscheuer et al., 2010).

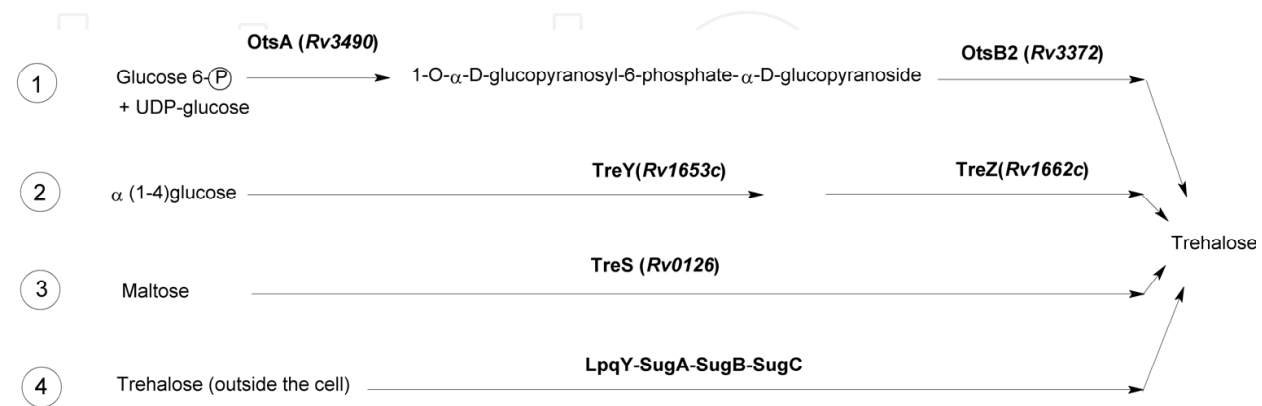


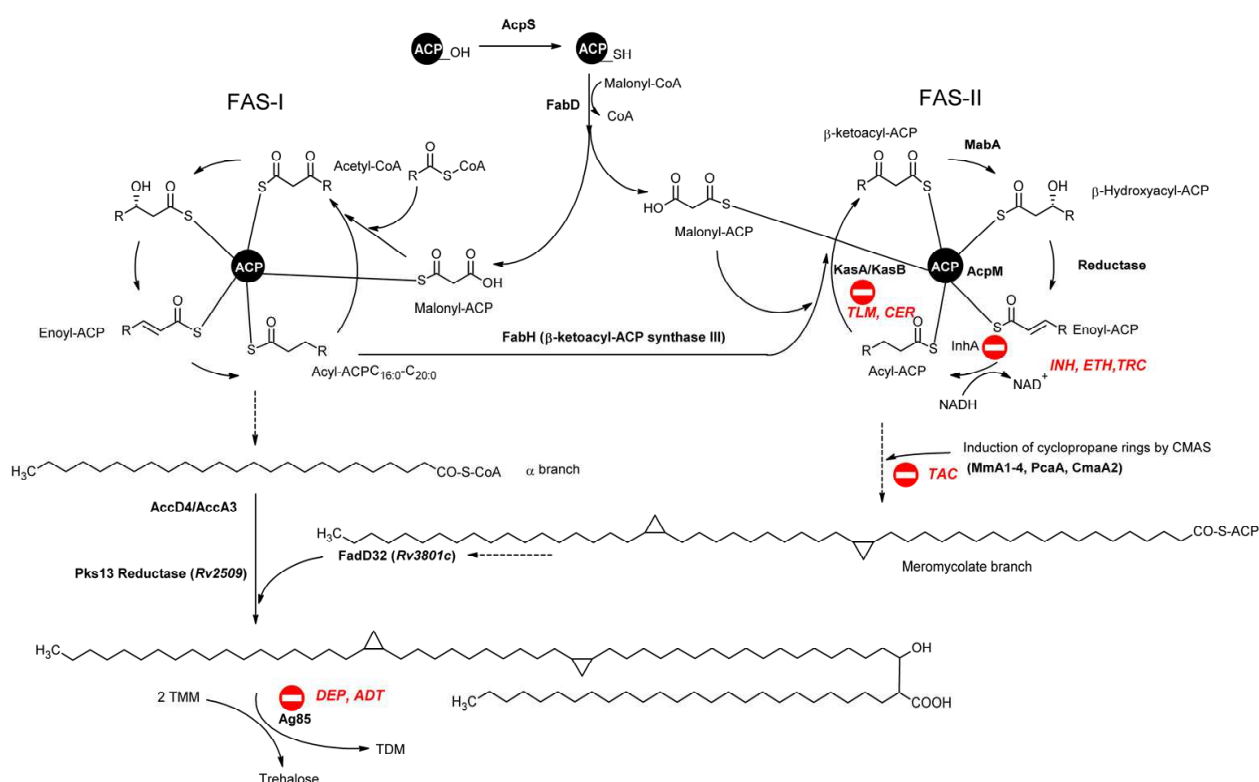
Fig. 2. Trehalose biosynthesis in *Mycobacterium tuberculosis*. 1, OtsAB pathway. 2, TreY-TreZ pathway. 3, TreS-pathway. 4, Trehalose import by an ATP-binding cassette transporter system.

3.3 Mycolic acid biosynthesis

Mycolic acids are β -hydroxy fatty acids with a long α -alkyl side chain. They are homologous series of fatty acids differing by a two-carbon unit (Asselineau & Lederer, 1950). The mycolic acids are composed of an α branch at the alpha position in respect to the carboxylic group and a meromycolate branch. The "short" α branch contains species dependent 20-26 saturated carbon atoms. The "long" meromycolate branch has 50-60 carbon atoms and its chemical composition is highly variable, containing cyclopropyl or unsaturated bonds (α -mycolates), methoxy (methoxymycolates) and keto (ketomycolates) groups (Alahari et al., 2007). The confusing denotation " α -mycolates" refers not to their position in the molecule but to their position on thin layer chromatography. The " α -mycolates" (cis, cis-dicyclopropyl fatty acids) are the most abundant mycolic acids in *M. tuberculosis* (~57%), followed by methoxymycolates (32%) and ketomycolates (11%). The methoxy- and ketomycolates can have either the cis or trans configuration on the proximal cyclopropane ring. In summary there are five main classes of mycolic acids in *M. tuberculosis* (Schroeder et al., 2002; Takayama et al., 2005).

In *M. tuberculosis* mycolic acids are essentially provided via conventional fatty acid biosynthesis. Mycobacteria contain both type I and type II FAS fatty acid biosynthesis systems. Fatty acid biosynthesis is initiated by the multifunctional FAS I enzyme (*Rv2524c*), catalyzing the de novo synthesis of long-chain acyl-CoAs (C16:0 and C18:0) from acetyl-CoA and using malonyl-CoA as an extender unit. The domains of the FAS-I multienzyme-complex are organized in the following order: acyltransferase, enoyl reductase, dehydratase, malonyl/palmitoyl transferase, acyl carrier protein (ACP), β -keto reductase, β -ketoacyl synthase (Fernandes & Kolattukudy, 1996). In *M. tuberculosis* the C16:0- and C18:0-S-ACP adducts, are converted either to the CoA derivatives or further elongated by FAS I to produce C26:0 (Kikuchi et al., 1992). In mycobacteria the de novo fatty acid biosynthesis is exclusively carried out by FAS-I, whereas the FAS-II system performs only the elongation of the fatty acids, generated by FAS-I. The FAS I and FAS II systems are connected by a key condensing enzyme, the β -ketoacyl ACP synthase III or *FabH*, which catalyzes a decarboxylative condensation of malonyl-ACP with the acyl-CoA (C16:0-C20:0) products of the FAS-I system (Fig. 3). The resulting 3-ketoacyl-ACP product is reduced to an acyl-ACP (extended by two carbons) and shuffled into the FAS II cycle.

The ACP cycles the growing acyl chain between four enzymes *MabA* (β -ketoacyl reductase), β -hydroxyacyl dehydratase, *InhA* (enoyl reductase) and *KasA/B* (β -ketoacyl synthase). *M. tuberculosis* contains two β -ketoacyl synthases, *KasA* and *KasB*, which share 67% identity. *KasA* seems to be essential for growth, while *KasB* is not essential but produces longer carbon chains (Bhatt et al., 2005; Slayden & Barry, 2002; Swanson et al., 2009). The deletion of *KasB* in *M. tuberculosis* leads to mycolic acids that are 2-6 carbons shorter in length and a defect in trans-cyclopropanation of oxygenated mycolic acids. Phenotypically leads a deletion of *KasB* to a loss of acid-fastness (Bhatt et al., 2007). The most potent inhibitor for mycolic acid biosynthesis is isoniazid (INH). INH is a prodrug which is converted to the isonicotinoyl radical by *KatG*. INH forms a covalent adduct with NAD. This INH-NAD adduct inhibits FAS-II enoyl-ACP reductase *InhA*, which in consequence leads to inhibition of mycolic acid biosynthesis, and ultimately to cell death (Mdluli et al., 1998; Takayama et al., 1972; 1975; Wilming & Johnsson, 1999). In *M. tuberculosis*, the C26:0



mycolyl- β -D-mannopyranosyl -1-phosphoheptaprenol (Myc-PL) (Besra et al., 1994). Myc-PL migrates to the inner surface of the cell membrane and docks next to an ABC transporter, with its hydrophobic heptaprenol tail. The mycolyl group is transferred to trehalose 6-phosphate by a proposed membrane-associated mycolyltransferase II to form TMM-phosphate, and the phosphate group is removed by the membrane-associated trehalose 6-phosphate phosphatase, yielding TMM. TMM is transported outside the cell by the ABC transporter (Fig. 4). There should be virtually no accumulation of TMM in the cytoplasm (Takayama et al., 2005).

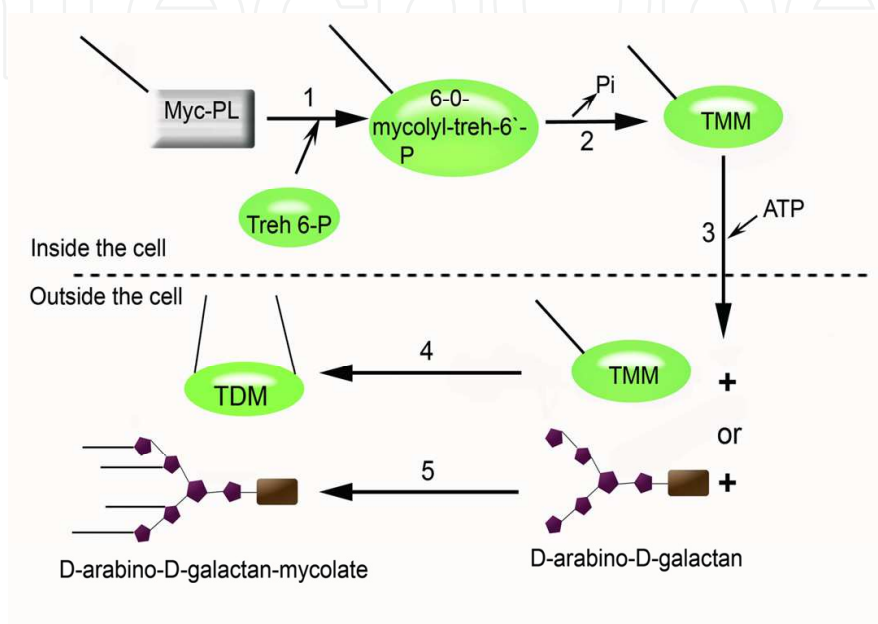


Fig. 4. The proposed process of incorporation of newly synthesized mycolic acids into major cell wall components. The process starts inside the cell. Newly synthesized mycolic acids are transferred to man-P-heptaprenol to produce 6-O-mycolyl- β -D-mannopyranosyl-1-phosphoheptaprenol (Myc-PL) and after that to trehalose 6-phosphate to yield TMM-P by the proposed membrane-associated mycolyltransferase II (reaction 1). TMM is produced by dephosphorylation of TMM-P by the membrane-bound TMM-P phosphatase (reaction 2). The transportation of TMM to the outside is catalyzed by a proposed ABC transporter cassette (TMM transporter) (reaction 3). Outside the cell the Ag85 complex catalyzes the transfer of mycolate to another TMM and arabinogalactan to yield TDM (reaction 4) or arabinogalactan-mycolate (reaction 5) (Takayama et al., 2005).

3.5 TDM biosynthesis by Ag85

The antigen 85 complex is composed of Ag85A (FbpA), Ag85B (FbpB), and Ag85C (FbpC) as the predominant secreted proteins in *M. tuberculosis*. The corresponding genes are *fbpA* (*Rv3804c*), *fbpB* (*Rv1886c*), and *fbpC* (*Rv0129c*) (Belisle et al., 1997; Wiker & Harboe, 1992). The 85 complex proteins share 68–80% sequence identity (Belisle et al., 1997; Ronning et al., 2004). The mycolyltransferases of the antigen 85 complex are located outside the cell membrane and transfer the lipid moiety of the glycolipid trehalose monomycolate (TMM) to another molecule of TMM yielding trehalose dimycolate or to arabinogalactan to form cell wall arabinogalactan-mycolate (Fig. 4) (Sanki et al., 2009a).

There is also evidence, the Ag85 complex proteins bind to fibronectin and the fibronectin-binding property of the Ag85 complex is important for mycobacterium life cycle in the host and macrophages (Klegerman et al., 1994; Ronning et al., 2004). The crystal structures of the three 30–32 kDa proteins (Ag85A, B and C) have been determined (Anderson et al., 2001; Ronning et al., 2004). These proteins contain a carboxylesterase domain bearing the highly-conserved consensus sequence GXXSXXG. The interaction between Ag85 and fibronectin is mediated by the sequence homologous to residues 56–66 (FEEYYQSGLSV) of the recombinant *M. tuberculosis* Ag85C (Ronning et al., 2004). Up to date, the question remains open why *Mycobacterium tuberculosis* has three antigen 85 enzymes sharing the similar sequence and substrate specificity (Daffe, 2000; Ronning et al., 2004).

Ag85 complex members from *M. tuberculosis* belong to the α/β hydrolase superfamily and catalyze the hydrolysis of ester and amide bonds using a catalytic triad comprised of Ser126, Glu230 and His262 in Ag85A/B and Ser124, Glu228 and His260 in Ag85C. All three enzymes contain two carbohydrate binding sites. The active site carbohydrate binding pocket binds TMM to form a temporary mycolate ester with the catalytic serine. The second carbohydrate binding site binds the incoming trehalose monomycolate, which “swings over” to the active site to displace the mycolate from its serine ester (Anderson et al., 2001; Ronning et al., 2004). The second trehalose binding site is separated from the acyl binding pocket by a bulky phenylalanine in Ag85A/B or a smaller leucine in Ag85C. All residues that form the active site carbohydrate binding pocket are 100% conserved in the *M. tuberculosis* antigen 85 proteins, while the surface of the acyl binding pocket, which is supposed to bind the long mycolate chains of TMM, exhibits slight differences. The conserved Leu152 in Ag85A and B is replaced by the bulky Phe150 in Ag85C, which in consequence leads to changes of surface topology in the mycolate binding portion (Fig. 5). The differences may alter substrate specificity and thus Ag85A, B and C might prefer different mycolic acids (Ronning et al., 2000; 2004).

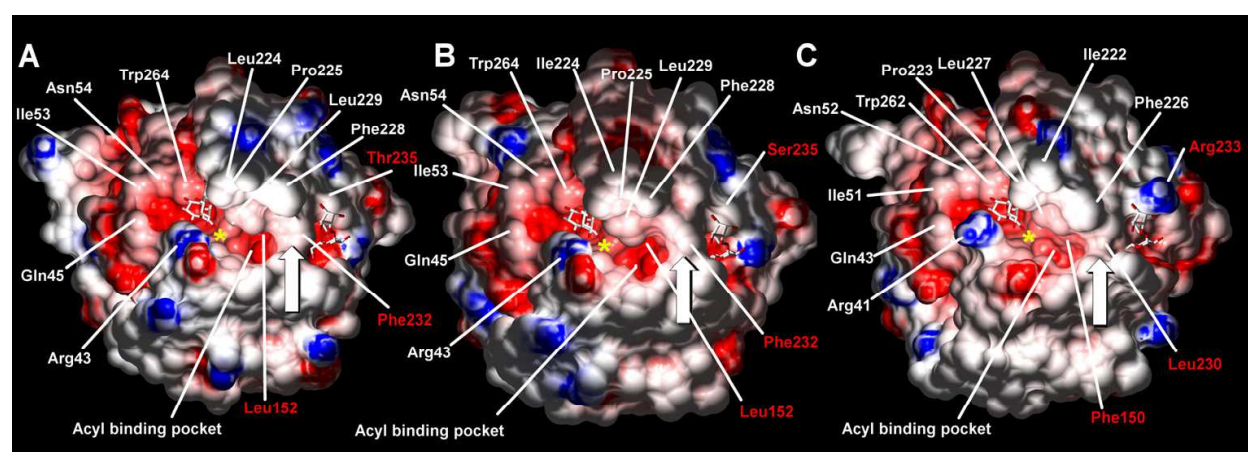


Fig. 5. Surface representation of Ag85A, B and C with two bound trehalose molecules. The trehalose molecules are depicted as ball-and-stick model. The position of the catalytic serine is indicated by a yellow asterisk. The carbohydrate binding pocket of all three proteins is 100% conserved (Arg43, Gln45, Ile53, Asn54, Trp264 in Ag85A and B) and Arg41, Gln43, Ile51, Asn52, Trp264 in Ag85C. Corresponding residues, which differ among the three proteins are shown with red labels. The separation of the second carbohydrate binding pocket from the acyl binding pocket by the Phe232 in Ag85A/B and the corresponding

smaller Leu230 in Ag85C is highlighted by an arrow. Also shown: Arginine 233, which covers the second carbohydrate binding pocket in Ag85C is replaced by smaller polar amino acids Thr235 and Ser235 in Ag85A and B, respectively. The surface is colored by electrostatic potential: The red and blue coloring represent negative and positive electrostatic potential, respectively. For Ag85A, B and C the coordinates from 1SFR, 1F0P and 1DQZ were used, respectively. The position of the trehalose molecules in Ag85A and Ag85C were modeled using the coordinates from 1F0P. The figure was prepared using GRASP (Nicholls et al., 1991).

3.6 Ag85 as a putative drug target for tuberculosis treatment

The ongoing treatment battle of tuberculosis is worsened by the emergence of new strains of *M. tuberculosis* which are resistant to standard antibiotics. In the urgent need of new targets the biogenesis of fatty acids, mycolic acids and glycolipids stay as hotspots. There is hope that the crystal structure of antigen 85A, 85B and 85C shall help in rational drug development for TB (Ronning et al., 2000; 2004).

The treatment by a trehalose analogue, 6-azido-6-deoxy- α,α' -trehalose (ADT) inhibited the activity of all members of Ag85 complex *in vitro* and the growth of *Mycobacterium aurum*, and it also increased the efficacy of various antibiotics, supporting the importance of TDM (Belisle et al., 1997; Mizuguchi et al., 1983). *M. tuberculosis* strain lacking Ag85C has a 40% decrease in the amount of cell wall linked mycolic acid, but with no change in the relative amounts of TMM and TDM (Jackson et al., 1999; Sanki et al., 2009a). Furthermore, an Ag85A knockout strain lost the ability to grow in macrophage-like cell-lines and poor media which highlights the role of Ag85A in virulence and survival of the organism (Armitage et al., 2000). In the last decades several antitubercular drugs have focused on targets in the mycobacterial cell wall (Johnson et al., 2006). Most commonly, ethambutol targets the synthesis of arabinogalactan. Isoniazid and ethionamide inhibit biosynthesis of mycolic acids (Johnson et al., 2006). Obviously, the crystal structure of antigen 85 complex is expected to accelerate the design of new drugs against Ag85 activity and cord factor biosynthesis (Table 1) (Gobec et al., 2004; Sanki et al., 2009b; Wang et al., 2004).

4. Drug development: Novel high-throughput screening assays for mycolyltransferase 85A

Since the protein/substrate interactions and co-crystal structure of Ag85 are now known, the search for rapid assays for high-throughput screening (HTS) of large substance libraries has increased considerably. Most of the mycolyltransferase assays previously published are not suitable for HTS, due to their complexity or use of radioactive substances. The first one is a widely used radioassay which monitors enzymatic transfer of mycolic acids from a lipid-soluble TMM molecule to a radioactive water-soluble trehalose. Manipulation of the radioactive products in a two-phase reaction, extraction and thin layer chromatography allows visualization of the products (Kremer et al., 2002; Sathyamoorthy & Takayama, 1987). Another test published uses the substrate analogue p-nitrophenyl-6-O-octanoyl-H-D-glucopyranoside that functions as the acyl donor but it may not represent the natural enzymatic activity (Boucau et al., 2009). Also an excess of D-glucose is added to the reaction to function as an acyl acceptor and to promote turnover of the enzyme. Recently new assay

for Ag85 was developed based on the use of mono and dihexanoyl trehalose substrates, followed by quantitation of the acyl-transfer to the unnatural trehalose by mass spectrometry (Backus et al., 2011).

Synthesis step	Enzyme	Compound/ class	References
FAS-I and FAS-II	KasA/KasB	Cerulenin (2R,3S-epoxy-4-oxo-7,10-trans,trans-dodecanoic acid amide	(Schroeder et al., 2002) (Johansson et al., 2008)
		TLM (Thiolactomycin)	(Douglas et al., 2002; Kremer et al., 2000; Luckner et al., 2010)
FAS-II	KasA/KasB	Platensimycin	(Brown et al., 2009)
		INH (Isoniazid)	(Slayden et al., 2000)
		ETH (Ethionamide)	(Kremer et al., 2003)
		TRC (Triclosan)	(McMurry et al., 1999)
	InhA	alkyl diphenyl ethers (Triclosan derivatives)	(Sullivan et al., 2006)
	InhA	2-(o-Tolyloxy)-5-hexylphenol (PT70)	(Luckner et al., 2010)
Mycolic acid biosynthesis	Unknown	N-octanesulfonylacetamide	(Parrish et al., 2001)
Cyclopropanation	CMASs (cmaA2, mmaA2 or pcaA)	TAC (Thiacetazone)	(Alahari et al., 2007)
	MmaA4	TAC (Thiacetazone)	(Alahari et al., 2007)
Cord-factor biosynthesis	Ag85C	Methyl β -D-arabinofuranoside	(Sanki et al., 2009b)
	Ag85C	5-S-alkyl-5-thio-arabinofuranoside analogues	(Sanki et al., 2008)
	Ag85 complex	Trehalose analogues	(Wang et al., 2004)
	Ag85 complex	ADT (6-azido-6-deoxy- α,α' -trehalose)	(Belisle et al., 1997; Mizuguchi et al., 1983)
	Ag85 complex	Phosphonate compounds	(Gobec et al., 2004; Gobec et al., 2007)
	Ag85C	DEP (Diethyl phosphate)	(Ronning et al., 2000)

Table 1. Inhibitors of cord factor biosynthesis

The mycobacterial glycolipids and TMM levels in the cell wall might give an indirect indication of the fitness of the cell inside the host cells specially in macrophages. Thus the quantitation of the mycobacterial TMM status after drug treatment may allow the estimation of drug effectiveness. Unfortunately, there is no method for measurement of the amount of glycolipids that is suitable for HTS. We designed an assay (Elamin et al., 2009) based on the use of natural substrate, and this mycolyltransferase assay offers a novel means to determine the TMM status of the mycobacterium cell wall and reflects the natural activity of mycolyltransferase enzyme based on simple steps. The new assay uses the natural substrate TMM, which can be easily purified from mycobacteria (Fujita et al.,

2005) and reflects natural activity, allowing to get the most accurate kinetic parameters. In the reaction Ag85A produces one molecule of trehalose as product per reaction cycle and by adding trehalase the trehalose converted to glucose, which can be easily measured (Fig. 6).

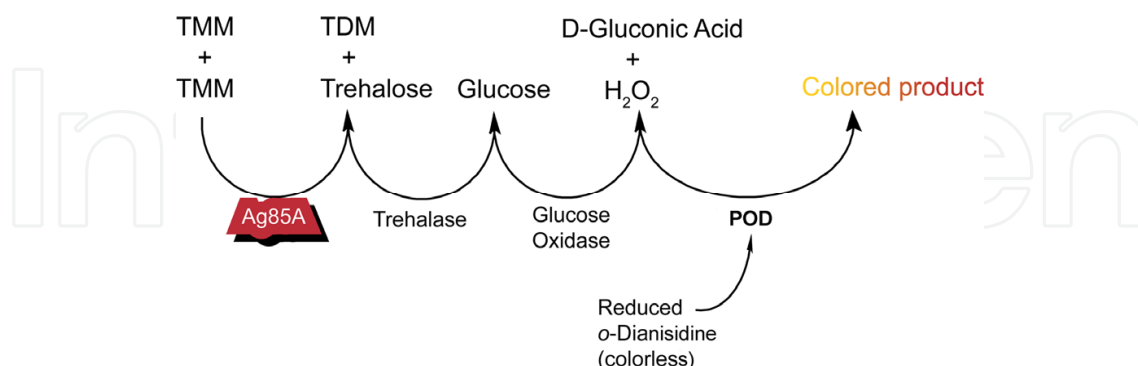


Fig. 6. Scheme for the new mycolyltransferase activity assay. Using trehalose that is produced as one of final products of mycolyltransferase reaction by trehalase to produce glucose, which is oxidized to gluconic acid and hydrogen peroxidase by glucose oxidase. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to produce a colored product (oxidized o-dianisidine), which will be converted to a stable colored product by sulfuric acid. The colored product is measured at 540 nm (Elamin et al., 2009).

Quantification of glucose is finally achieved by the glucose oxidase assay (Washko & Rice, 1961). The amount of glucose is proportional to the TMM concentration. The assay showed that the antigen 85A can be assayed in the presence of methanol or mixture of chloroform/methanol, which is usually used to extract and purify the glycolipids from mycobacterium cell wall fractions. The results from substrate/solvent experiments showed that the enzyme activity was reduced in the presence of organic solvents than in standard buffer reaction alone. This indicates and proves that this method is useful to quantify the TMM from the total lipid of mycobacterium cells.

One molecule of trehalose produced from TMM processed by Ag85 complex, which by our method is converted to two molecules of glucose. We can calculate the original concentration of TMM and concentration of TDM and trehalose in the reaction by the following equations:

$$\text{Concentration of [TMM]} = \text{concentration of [glucose]} \quad (1)$$

$$\text{Concentration of [TDM]} = \text{concentration of [glucose]}/2 \quad (2)$$

$$\text{Concentration of [trehalose]} = \text{concentration of [glucose]}/2 \quad (3)$$

One has to keep in mind that the extracted total lipids contain free trehalose and glucose and will affect the final calculations. In this case and to calculate the TMM concentration in total lipids one should run different negative controls. The Z' factor (Zhang et al., 1999) measurement of the current assay in different volumes indicates an excellent signal/noise (S/N) ratio for the assay and its high potential for HTS applications (Table 2).

Reaction volume	200 µl	300 µl	350 µl
Z' factor	0.67 ±0.021	0.72 ± 0.014	0.73 ±0.012

Table 2. The calculated Z' factor at different volumes from 96-well plate format assays.

5. Concluding remarks

Large gaps remain in our understanding of mycobacterium pathogenesis and persistence including the critical questions how bacteria survive in host cells and escape from the therapy. Future work on mycobacterial cell wall biosynthesis especially glycolipids and related pathways is expected to reveal *in vivo* drug-resistance mechanism. Perhaps more notably, the described new and low-cost colorimetric method based on use of TMM as natural substrate could brings flexibility and convenience in HT-screening of substance libraries and help in the development of novel drugs against tuberculosis.

6. References

Alahari, A.;Trivelli, X.;Guerardel, Y.;Dover, L.G.;Besra, G.S.;Sacchettini, J.C.;Reynolds, R.C.;Coxon, G.D., & Kremer, L. (2007) Thiacetazone, an antitubercular drug that inhibits cyclopropanation of cell wall mycolic acids in mycobacteria. *PLoS One*, Vol.2, No.12, (December 2007), pp. e1343, ISSN 1932-6203

Alahari, A.;Alibaud, L.;Trivelli, X.;Gupta, R.;Lamichhane, G.;Reynolds, R.C.;Bishai, W.R.;Guerardel, Y., & Kremer, L. (2009) Mycolic acid methyltransferase, MmaA4, is necessary for thiacetazone susceptibility in *Mycobacterium tuberculosis*. *Molecular microbiology*, Vol.71, No.5, (March 2009), pp. 1263-1277, ISSN 1365-2958

Anderson, D.H.;Harth, G.;Horwitz, M.A., & Eisenberg, D. (2001) An interfacial mechanism and a class of inhibitors inferred from two crystal structures of the *Mycobacterium tuberculosis* 30 kDa major secretory protein (Antigen 85B), a mycolyl transferase. *Journal of molecular biology*, Vol.307, No.2, (March 2001), pp. 671-681, ISSN 0022-2836

Armitige, L.Y.;Jagannath, C.;Wanger, A.R., & Norris, S.J. (2000) Disruption of the genes encoding antigen 85A and antigen 85B of *Mycobacterium tuberculosis* H37Rv: effect on growth in culture and in macrophages. *Infection and immunity*, Vol.68, No.2, (February 2000), pp. 767-778, ISSN 0019-9567

Asselineau, J., & Lederer, E. (1950) Structure of the mycolic acids of Mycobacteria. *Nature*, Vol.166, No.4227, (November 1950), pp. 782-783, ISSN 0028-0836

Backus, K.M.;Boshoff, H.I.;Barry, C.S.;Boutureira, O.;Patel, M.K.;D'Hooge, F.;Lee, S.S.;Via, L.E.;Tahlan, K.;Barry, C.E., 3rd, & Davis, B.G. (2011) Uptake of unnatural trehalose analogs as a reporter for *Mycobacterium tuberculosis*. *Nature chemical biology*, Vol.7, No.4, (April 2011), pp. 228-235, ISSN 1552-4469

Barry, C.E., 3rd;Lee, R.E.;Mdluli, K.;Sampson, A.E.;Schroeder, B.G.;Slayden, R.A., & Yuan, Y. (1998) Mycolic acids: structure, biosynthesis and physiological functions. *Progress in lipid research*, Vol.37, No.2-3, (July-August 1998), pp. 143-179, ISSN 0163-7827

- Behling, C.A.;Bennett, B.;Takayama, K., & Hunter, R.L. (1993a) Development of a trehalose 6,6'-dimycolate model which explains cord formation by *Mycobacterium tuberculosis*. *Infection and immunity*, Vol.61, No.6, (June 1993a), pp. 2296-2303, ISSN 0019-9567
- Behling, C.A.;Perez, R.L.;Kidd, M.R.;Staton, G.W., Jr., & Hunter, R.L. (1993b) Induction of pulmonary granulomas, macrophage procoagulant activity, and tumor necrosis factor-alpha by trehalose glycolipids. *Annals of clinical and laboratory science*, Vol.23, No.4, (July-August 1993b), pp. 256-266, ISSN 0091-7370
- Bekierkunst, A.;Levij, I.S.;Yarkoni, E.;Vilkas, E.;Adam, A., & Lederer, E. (1969) Granuloma formation induced in mice by chemically defined mycobacterial fractions. *Journal of bacteriology*, Vol.100, No.1, (October 1969), pp. 95-102, ISSN 0021-9193
- Belisle, J.T.;Vissa, V.D.;Sievert, T.;Takayama, K.;Brennan, P.J., & Besra, G.S. (1997) Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science (New York, N.Y.)*, Vol.276, No.5317, (May 1997), pp. 1420-1422, ISSN 0036-8075
- Besra, G.S.;Sievert, T.;Lee, R.E.;Slayden, R.A.;Brennan, P.J., & Takayama, K. (1994) Identification of the apparent carrier in mycolic acid synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.91, No.26, (December 1994), pp. 12735-12739, ISSN 0027-8424
- Bhatt, A.;Kremer, L.;Dai, A.Z.;Sacchetti, J.C., & Jacobs, W.R., Jr. (2005) Conditional depletion of KasA, a key enzyme of mycolic acid biosynthesis, leads to mycobacterial cell lysis. *Journal of bacteriology*, Vol.187, No.22, (November 2005), pp. 7596-7606, ISSN 0021-9193
- Bhatt, A.;Fujiwara, N.;Bhatt, K.;Gurcha, S.S.;Kremer, L.;Chen, B.;Chan, J.;Porcelli, S.A.;Kobayashi, K.;Besra, G.S., & Jacobs, W.R., Jr. (2007) Deletion of *kasB* in *Mycobacterium tuberculosis* causes loss of acid-fastness and subclinical latent tuberculosis in immunocompetent mice. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.104, No.12, (March 2007), pp. 5157-5162, ISSN 0027-8424
- Bloch, H. (1950) Studies on the virulence of tubercle bacilli; isolation and biological properties of a constituent of virulent organisms. *The Journal of experimental medicine*, Vol.91, No.2, (February 1950), pp. 197-218, pl, ISSN 0022-1007
- Boucau, J.;Sanki, A.K.;Voss, B.J.;Sucheck, S.J., & Ronning, D.R. (2009) A coupled assay measuring *Mycobacterium tuberculosis* antigen 85C enzymatic activity. *Analytical biochemistry*, Vol.385, No.1, (February 2009), pp. 120-127, ISSN 1096-0309
- Brennan, P.J., & Nikaido, H. (1995) The envelope of mycobacteria. *Annual review of biochemistry*, Vol.64, (July 1995), pp. 29-63, ISSN 0066-4154
- Brennan, P.J. (2003) Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. *Tuberculosis (Edinburgh, Scotland)*, Vol.83, No.1-3, (February 2003), pp. 91-97, ISSN 1472-9792
- Brown, A.K.;Taylor, R.C.;Bhatt, A.;Futterer, K., & Besra, G.S. (2009) Platensimycin activity against mycobacterial beta-ketoacyl-ACP synthases. *PLoS One*, Vol.4, No.7, (July 2009), pp. e6306, ISSN 1932-6203
- Carroll, P.;Faray-Kele, M.C., & Parish, T. (2011) Identifying Vulnerable Pathways in *Mycobacterium tuberculosis* by Using a Knockdown Approach. *Applied and*

- environmental microbiology*, Vol.77, No.14, (July 2011), pp. 5040-5043, ISSN 1098-5336
- Casadevall, A., & Pirofski, L.A. (2000) Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. *Infect Immun*, Vol.68, No.12, (December 2000), pp. 6511-6518, ISSN 0019-9567
- Coelho, E.A.;Tavares, C.A.;Lima Kde, M.;Silva, C.L.;Rodrigues, J.M., Jr., & Fernandes, A.P. (2006) *Mycobacterium* hsp65 DNA entrapped into TDM-loaded PLGA microspheres induces protection in mice against *Leishmania (Leishmania) major* infection. *Parasitology research*, Vol.98, No.6, (May 2006), pp. 568-575, ISSN 0932-0113
- Coker, R.J. (2004) Review: multidrug-resistant tuberculosis: public health challenges. *Tropical medicine & international health : TM & IH*, Vol.9, No.1, (January 2004), pp. 25-40, ISSN 1360-2276
- Daffe, M. (2000) The mycobacterial antigens 85 complex - from structure to function and beyond. *Trends in microbiology*, Vol.8, No.10, (October 2000), pp. 438-440, ISSN 0966-842X
- Davidson, J.;Rosenkrands, I.;Christensen, D.;Vangala, A.;Kirby, D.;Perrie, Y.;Agger, E.M., & Andersen, P. (2005) Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from *M. tuberculosis* (trehalose 6,6'-dibehenate)-a novel adjuvant inducing both strong CMI and antibody responses. *Biochimica et biophysica acta*, Vol.1718, No.1-2, (December 2005), pp. 22-31, ISSN 0006-3002
- Douglas, J.D.;Senior, S.J.;Morehouse, C.;Phetsukiri, B.;Campbell, I.B.;Besra, G.S., & Minnikin, D.E. (2002) Analogues of thiolactomycin: potential drugs with enhanced anti-mycobacterial activity. *Microbiology (Reading, England)*, Vol.148, No.Pt 10, (October 2002), pp. 3101-3109, ISSN 1350-0872
- Elamin, A.A.;Stehr, M.;Oehlmann, W., & Singh, M. (2009) The mycolyltransferase 85A, a putative drug target of *Mycobacterium tuberculosis*: development of a novel assay and quantification of glycolipid-status of the mycobacterial cell wall. *Journal of microbiological methods*, Vol.79, No.3, (December 2009), pp. 358-363, ISSN 1872-8359
- Elbein, A.D., & Mitchell, M. (1973) Levels of glycogen and trehalose in *Mycobacterium smegmatis* and the purification and properties of the glycogen synthetase. *Journal of bacteriology*, Vol.113, No.2, (February 1973), pp. 863-873, ISSN 0021-9193
- Elbein, A.D.;Pan, Y.T.;Pastuszak, I., & Carroll, D. (2003) New insights on trehalose: a multifunctional molecule. *Glycobiology*, Vol.13, No.4, (April 2003), pp. 17R-27R, ISSN 0959-6658
- Fernandes, N.D., & Kolattukudy, P.E. (1996) Cloning, sequencing and characterization of a fatty acid synthase-encoding gene from *Mycobacterium tuberculosis* var. *bovis* BCG. *Gene*, Vol.170, No.1, (April 1996), pp. 95-99, ISSN 0378-1119
- Fujita, Y.;Naka, T.;Doi, T., & Yano, I. (2005) Direct molecular mass determination of trehalose monomycolate from 11 species of mycobacteria by MALDI-TOF mass spectrometry. *Microbiology (Reading, England)*, Vol.151, No.Pt 5, (May 2005), pp. 1443-1452, ISSN 1350-0872
- Gavalda, S.;Leger, M.;van der Rest, B.;Stella, A.;Bardou, F.;Montrozier, H.;Chalut, C.;Burlet-Schiltz, O.;Marrakchi, H.;Daffe, M., & Quemard, A. (2009) The Pks13/FadD32

- crosstalk for the biosynthesis of mycolic acids in *Mycobacterium tuberculosis*. *The Journal of biological chemistry*, Vol.284, No.29, (July 2009), pp. 19255-19264, ISSN 0021-9258
- Glickman, M.S.;Cox, J.S., & Jacobs, W.R., Jr. (2000) A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of *Mycobacterium tuberculosis*. *Molecular cell*, Vol.5, No.4, (April 2000), pp. 717-727, ISSN 1097-2765
- Gobec, S.;Plantan, I.;Mravljak, J.;Wilson, R.A.;Besra, G.S., & Kikelj, D. (2004) Phosphonate inhibitors of antigen 85C, a crucial enzyme involved in the biosynthesis of the *Mycobacterium tuberculosis* cell wall. *Bioorganic & medicinal chemistry letters*, Vol.14, No.13, (July 2004), pp. 3559-3562, ISSN 0960-894X
- Gobec, S.;Plantan, I.;Mravljak, J.;Svajger, U.;Wilson, R.A.;Besra, G.S.;Soares, S.L.;Appelberg, R., & Kikelj, D. (2007) Design, synthesis, biochemical evaluation and antimycobacterial action of phosphonate inhibitors of antigen 85C, a crucial enzyme involved in biosynthesis of the mycobacterial cell wall. *European journal of medicinal chemistry*, Vol.42, No.1, (January 2007), pp. 54-63, ISSN 0223-5234
- Hunter, R.L.;Olsen, M.;Jagannath, C., & Actor, J.K. (2006a) Trehalose 6,6'-dimycolate and lipid in the pathogenesis of caseating granulomas of tuberculosis in mice. *The American journal of pathology*, Vol.168, No.4, (April 2006a), pp. 1249-1261, ISSN 0002-9440
- Hunter, R.L.;Olsen, M.R.;Jagannath, C., & Actor, J.K. (2006b) Multiple roles of cord factor in the pathogenesis of primary, secondary, and cavitary tuberculosis, including a revised description of the pathology of secondary disease. *Annals of clinical and laboratory science*, Vol.36, No.4, (Autumn 2006b), pp. 371-386, ISSN 0091-7370
- Indrigo, J.;Hunter, R.L., Jr., & Actor, J.K. (2002) Influence of trehalose 6,6'-dimycolate (TDM) during mycobacterial infection of bone marrow macrophages. *Microbiology (Reading, England)*, Vol.148, No.Pt 7, (July 2002), pp. 1991-1998, ISSN 1350-0872
- Ishikawa, E.;Ishikawa, T.;Morita, Y.S.;Toyonaga, K.;Yamada, H.;Takeuchi, O.;Kinoshita, T.;Akira, S.;Yoshikai, Y., & Yamasaki, S. (2009) Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *The Journal of experimental medicine*, Vol.206, No.13, (December 2009), pp. 2879-2888, ISSN 1540-9538
- Jackson, M.;Raynaud, C.;Laneelle, M.A.;Guilhot, C.;Laurent-Winter, C.;Ensergueix, D.;Gicquel, B., & Daffe, M. (1999) Inactivation of the antigen 85C gene profoundly affects the mycolate content and alters the permeability of the *Mycobacterium tuberculosis* cell envelope. *Molecular microbiology*, Vol.31, No.5, (March 1999), pp. 1573-1587, ISSN 0950-382X
- Johansson, P.;Wiltschi, B.;Kumari, P.;Kessler, B.;Vonnrhein, C.;Vonck, J.;Oesterheld, D., & Grininger, M. (2008) Inhibition of the fungal fatty acid synthase type I multienzyme complex. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.105, No.35, (September 2008), pp. 12803-12808, ISSN 1091-6490
- Johnson, R.;Streicher, E.M.;Louw, G.E.;Warren, R.M.;van Helden, P.D., & Victor, T.C. (2006) Drug resistance in *Mycobacterium tuberculosis*. *Curr Issues Mol Biol*, Vol.8, No.2, (July 2006), pp. 97-111, ISSN 1467-3037

- Kalscheuer, R.;Weinrick, B.;Veeraraghavan, U.;Besra, G.S., & Jacobs, W.R., Jr. (2010) Trehalose-recycling ABC transporter LpqY-SugA-SugB-SugC is essential for virulence of *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.107, No.50, (December 2010), pp. 21761-21766, ISSN 1091-6490
- Kaur, D.;Guerin, M.E.;Skovierova, H.;Brennan, P.J., & Jackson, M. (2009) Chapter 2: Biogenesis of the cell wall and other glycoconjugates of *Mycobacterium tuberculosis*. *Advances in applied microbiology*, Vol.69, (September 2009), pp. 23-78, ISSN 0065-2164
- Kikuchi, S.;Rainwater, D.L., & Kolattukudy, P.E. (1992) Purification and characterization of an unusually large fatty acid synthase from *Mycobacterium tuberculosis* var. *bovis* BCG. *Archives of biochemistry and biophysics*, Vol.295, No.2, (June 1992), pp. 318-326, ISSN 0003-9861
- Kilburn, J.O.;Takayama, K., & Armstrong, E.L. (1982) Synthesis of trehalose dimycolate (cord factor) by a cell-free system of *Mycobacterium smegmatis*. *Biochemical and biophysical research communications*, Vol.108, No.1, (September 1982), pp. 132-139, ISSN 0006-291X
- Klegerman, M.E.;Oner, F.;Morris, P.;Son, K., & Groves, M.J. (1994) Isolation of a fibronectin-binding tryptic peptide from the antigen 85A protein of *Mycobacterium bovis* BCG. *Microbios*, Vol.80, No.324, (1994), pp. 173-180, ISSN 0026-2633
- Koch, R. (1884) Die aetiologie der tuberkulose. *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, Vol.2, 1884), pp. 1-88,
- Kremer, L.;Douglas, J.D.;Baulard, A.R.;Morehouse, C.;Guy, M.R.;Alland, D.;Dover, L.G.;Lakey, J.H.;Jacobs, W.R., Jr.;Brennan, P.J.;Minnikin, D.E., & Besra, G.S. (2000) Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting KasA and KasB condensing enzymes in *Mycobacterium tuberculosis*. *J Biol Chem*, Vol.275, No.22, (June 2000), pp. 16857-16864, ISSN 0021-9258
- Kremer, L.;Maughan, W.N.;Wilson, R.A.;Dover, L.G., & Besra, G.S. (2002) The *M. tuberculosis* antigen 85 complex and mycolyltransferase activity. *Lett Appl Microbiol.*, Vol.34, No.4, (April 2002), pp. 233-237.,
- Kremer, L.;Dover, L.G.;Morbidity, H.R.;Vilcheze, C.;Maughan, W.N.;Baulard, A.;Tu, S.C.;Honore, N.;Deretic, V.;Sacchetti, J.C.;Locht, C.;Jacobs, W.R., Jr., & Besra, G.S. (2003) Inhibition of InhA activity, but not KasA activity, induces formation of a KasA-containing complex in mycobacteria. *The Journal of biological chemistry*, Vol.278, No.23, (June 2003), pp. 20547-20554, ISSN 0021-9258
- Lima, K.M.;Santos, S.A.;Lima, V.M.;Coelho-Castelo, A.A.;Rodrigues, J.M., Jr., & Silva, C.L. (2003) Single dose of a vaccine based on DNA encoding mycobacterial hsp65 protein plus TDM-loaded PLGA microspheres protects mice against a virulent strain of *Mycobacterium tuberculosis*. *Gene therapy*, Vol.10, No.8, (April 2003), pp. 678-685, ISSN 0969-7128
- Luckner, S.R.;Liu, N.;am Ende, C.W.;Tonge, P.J., & Kisker, C. (2010) A slow, tight binding inhibitor of InhA, the enoyl-acyl carrier protein reductase from *Mycobacterium tuberculosis*. *The Journal of biological chemistry*, Vol.285, No.19, (May 2010), pp. 14330-14337, ISSN 1083-351X

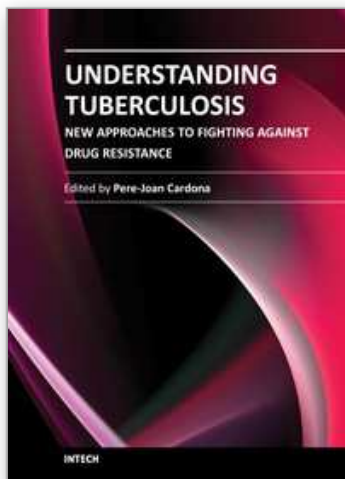
- Matsunaga, I., & Moody, D.B. (2009) Mincle is a long sought receptor for mycobacterial cord factor. *J Exp Med*, Vol.206, No.13, (December 2009), pp. 2865-2868, ISSN 1540-9538
- McMurry, L.M.;McDermott, P.F., & Levy, S.B. (1999) Genetic evidence that InhA of *Mycobacterium smegmatis* is a target for triclosan. *Antimicrobial agents and chemotherapy*, Vol.43, No.3, (March 1999), pp. 711-713, ISSN 0066-4804
- Mdluli, K.;Slayden, R.A.;Zhu, Y.;Ramaswamy, S.;Pan, X.;Mead, D.;Crane, D.D.;Musser, J.M., & Barry, C.E., 3rd (1998) Inhibition of a *Mycobacterium tuberculosis* beta-ketoacyl ACP synthase by isoniazid. *Science (New York, N.Y.)*, Vol.280, No.5369, (June 1998), pp. 1607-1610, ISSN 0036-8075
- Middlebrook, G.;Dubos, R.J., & Pierce, C. (1947) Virulence and Morphological Characteristics of Mammalian Tubercle Bacilli. *The Journal of experimental medicine*, Vol.86, No.2, (July 1947), pp. 175-184, ISSN 0022-1007
- Mizuguchi, Y.;Udou, T., & Yamada, T. (1983) Mechanism of antibiotic resistance in *Mycobacterium intracellulare*. *Microbiology and immunology*, Vol.27, No.5, (1983), pp. 425-431, ISSN 0385-5600
- Murphy, H.N.;Stewart, G.R.;Mischenko, V.V.;Apt, A.S.;Harris, R.;McAlister, M.S.;Driscoll, P.C.;Young, D.B., & Robertson, B.D. (2005) The OtsAB pathway is essential for trehalose biosynthesis in *Mycobacterium tuberculosis*. *The Journal of biological chemistry*, Vol.280, No.15, (April 2005), pp. 14524-14529, ISSN 0021-9258
- Nicholls, A.;Sharp, K.A., & Honig, B. (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins*, Vol.11, No.4, (December 1991), pp. 281-296, ISSN 0887-3585
- Noll, H., H. Bloch, J. Asselineau, and E. Lederer (1956) The chemical structure of the cord factor of *Mycobacterium tuberculosis*. *Biochim. Biophys. Acta*, Vol.20, (1956), pp. 299-318,
- Oswald, I.P.;Dozois, C.M.;Petit, J.F., & Lemaire, G. (1997) Interleukin-12 synthesis is a required step in trehalose dimycolate-induced activation of mouse peritoneal macrophages. *Infection and immunity*, Vol.65, No.4, (April 1997), pp. 1364-1369, ISSN 0019-9567
- Pan, Y.T.;Carroll, J.D., & Elbein, A.D. (2002) Trehalose-phosphate synthase of *Mycobacterium tuberculosis*. Cloning, expression and properties of the recombinant enzyme. *European journal of biochemistry / FEBS*, Vol.269, No.24, (December 2002), pp. 6091-6100, ISSN 0014-2956
- Parrish, N.M.;Houston, T.;Jones, P.B.;Townsend, C., & Dick, J.D. (2001) In vitro activity of a novel antimycobacterial compound, N-octanesulfonylacetamide, and its effects on lipid and mycolic acid synthesis. *Antimicrobial agents and chemotherapy*, Vol.45, No.4, (April 2001), pp. 1143-1150, ISSN 0066-4804
- Perez, R.L.;Roman, J.;Staton, G.W., Jr., & Hunter, R.L. (1994) Extravascular coagulation and fibrinolysis in murine lung inflammation induced by the mycobacterial cord factor trehalose-6,6'-dimycolate. *American journal of respiratory and critical care medicine*, Vol.149, No.2 Pt 1, (February 1994), pp. 510-518, ISSN 1073-449X
- Perez, R.L.;Roman, J.;Roser, S.;Little, C.;Olsen, M.;Indrigo, J.;Hunter, R.L., & Actor, J.K. (2000) Cytokine message and protein expression during lung granuloma formation and resolution induced by the mycobacterial cord factor trehalose-6,6'-dimycolate.

- Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*, Vol.20, No.9, (September 2000), pp. 795-804, ISSN 1079-9907
- Portevin, D.;De Sousa-D'Auria, C.;Houssin, C.;Grimaldi, C.;Chami, M.;Daffe, M., & Guilhot, C. (2004) A polyketide synthase catalyzes the last condensation step of mycolic acid biosynthesis in mycobacteria and related organisms. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.101, No.1, (January 2004), pp. 314-319, ISSN 0027-8424
- Portevin, D.;de Sousa-D'Auria, C.;Montrozier, H.;Houssin, C.;Stella, A.;Laneelle, M.A.;Bardou, F.;Guilhot, C., & Daffe, M. (2005) The acyl-AMP ligase FadD32 and AccD4-containing acyl-CoA carboxylase are required for the synthesis of mycolic acids and essential for mycobacterial growth: identification of the carboxylation product and determination of the acyl-CoA carboxylase components. *The Journal of biological chemistry*, Vol.280, No.10, (March 2005), pp. 8862-8874, ISSN 0021-9258
- Ronning, D.R.;Klabunde, T.;Besra, G.S.;Vissa, V.D.;Belisle, J.T., & Sacchettini, J.C. (2000) Crystal structure of the secreted form of antigen 85C reveals potential targets for mycobacterial drugs and vaccines. *Nature structural biology*, Vol.7, No.2, (February 2000), pp. 141-146, ISSN 1072-8368
- Ronning, D.R.;Vissa, V.;Besra, G.S.;Belisle, J.T., & Sacchettini, J.C. (2004) *Mycobacterium tuberculosis* antigen 85A and 85C structures confirm binding orientation and conserved substrate specificity. *The Journal of biological chemistry*, Vol.279, No.35, (August 2004), pp. 36771-36777, ISSN 0021-9258
- Russell, D.G.;Barry, C.E., 3rd, & Flynn, J.L. (2010) Tuberculosis: what we don't know can, and does, hurt us. *Science (New York, N.Y.)*, Vol.328, No.5980, (May 2010), pp. 852-856, ISSN 1095-9203
- Ryll, R.;Kumazawa, Y., & Yano, I. (2001) Immunological properties of trehalose dimycolate (cord factor) and other mycolic acid-containing glycolipids--a review. *Microbiology and immunology*, Vol.45, No.12, 2001), pp. 801-811, 0385-5600
- Saito, R.;Tanaka, A.;Sugiyama, K.;Azuma, I., & Yamamura, Y. (1976) Adjuvant effect of cord factor, a mycobacterial lipid. *Infection and immunity*, Vol.13, No.3, (March 1976), pp. 776-781, ISSN 0019-9567
- Sanki, A.K.;Boucau, J.;Srivastava, P.;Adams, S.S.;Ronning, D.R., & Sucheck, S.J. (2008) Synthesis of methyl 5-S-alkyl-5-thio-D-arabinofuranosides and evaluation of their antimycobacterial activity. *Bioorganic & medicinal chemistry*, Vol.16, No.10, (May 2008), pp. 5672-5682, ISSN 1464-3391
- Sanki, A.K.;Boucau, J.;Ronning, D.R., & Sucheck, S.J. (2009a) Antigen 85C-mediated acyl-transfer between synthetic acyl donors and fragments of the arabinan. *Glycoconjugate journal*, Vol.26, No.5, (July 2009a), pp. 589-596, ISSN 1573-4986
- Sanki, A.K.;Boucau, J.;Umesiri, F.E.;Ronning, D.R., & Sucheck, S.J. (2009b) Design, synthesis and biological evaluation of sugar-derived esters, alpha-ketoesters and alpha-ketoamides as inhibitors for *Mycobacterium tuberculosis* antigen 85C. *Molecular bioSystems*, Vol.5, No.9, (September 2009b), pp. 945-956, ISSN 1742-2051

- Sathyamoorthy, N., & Takayama, K. (1987) Purification and characterization of a novel mycolic acid exchange enzyme from *Mycobacterium smegmatis*. *The Journal of biological chemistry*, Vol.262, No.28, (October 1987), pp. 13417-13423, ISSN 0021-9258
- Schroeder, E.K.;de Souza, N.;Santos, D.S.;Blanchard, J.S., & Basso, L.A. (2002) Drugs that inhibit mycolic acid biosynthesis in *Mycobacterium tuberculosis*. *Curr Pharm Biotechnol*, Vol.3, No.3, (September 2002), pp. 197-225, ISSN 1389-2010
- Silva, C.L., & Faccioli, L.H. (1988) Tumor necrosis factor (cachectin) mediates induction of cachexia by cord factor from mycobacteria. *Infection and immunity*, Vol.56, No.12, (December 1988), pp. 3067-3071, ISSN 0019-9567
- Slayden, R.A.;Lee, R.E., & Barry, C.E., 3rd (2000) Isoniazid affects multiple components of the type II fatty acid synthase system of *Mycobacterium tuberculosis*. *Molecular microbiology*, Vol.38, No.3, (November 2000), pp. 514-525, ISSN 0950-382X
- Slayden, R.A., & Barry, C.E., 3rd (2002) The role of KasA and KasB in the biosynthesis of meromycolic acids and isoniazid resistance in *Mycobacterium tuberculosis*. *Tuberculosis (Edinburgh, Scotland)*, Vol.82, No.4, (August 2002), pp. 149-160, ISSN 1472-9792
- Spargo, B.J.;Crowe, L.M.;Ioned, T.;Beaman, B.L., & Crowe, J.H. (1991) Cord factor (alpha,alpha-trehalose 6,6'-dimycolate) inhibits fusion between phospholipid vesicles. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.88, No.3, (February 1991), pp. 737-740, ISSN 0027-8424
- Sullivan, T.J.;Truglio, J.J.;Boyne, M.E.;Novichenok, P.;Zhang, X.;Stratton, C.F.;Li, H.J.;Kaur, T.;Amin, A.;Johnson, F.;Slayden, R.A.;Kisker, C., & Tonge, P.J. (2006) High affinity InhA inhibitors with activity against drug-resistant strains of *Mycobacterium tuberculosis*. *ACS chemical biology*, Vol.1, No.1, (February 2006), pp. 43-53, ISSN 1554-8937
- Swanson, S.;Gokulan, K., & Sacchettini, J.C. (2009) KasA, another brick in the mycobacterial cell wall. *Structure (London, England : 1993)*, Vol.17, No.7, (July 2009), pp. 914-915, ISSN 1878-4186
- Takayama, K.;Wang, L., & David, H.L. (1972) Effect of isoniazid on the in vivo mycolic acid synthesis, cell growth, and viability of *Mycobacterium tuberculosis*. *Antimicrobial agents and chemotherapy*, Vol.2, No.1, (July 1972), pp. 29-35, ISSN 0066-4804
- Takayama, K.;Schnoes, H.K.;Armstrong, E.L., & Boyle, R.W. (1975) Site of inhibitory action of isoniazid in the synthesis of mycolic acids in *Mycobacterium tuberculosis*. *Journal of lipid research*, Vol.16, No.4, (July 1975), pp. 308-317, ISSN 0022-2275
- Takayama, K.;Wang, C., & Besra, G.S. (2005) Pathway to synthesis and processing of mycolic acids in *Mycobacterium tuberculosis*. *Clinical microbiology reviews*, Vol.18, No.1, (January 2005), pp. 81-101, ISSN 0893-8512
- Wang, J.;Elchert, B.;Hui, Y.;Takemoto, J.Y.;Bensaci, M.;Wennergren, J.;Chang, H.;Rai, R., & Chang, C.W. (2004) Synthesis of trehalose-based compounds and their inhibitory activities against *Mycobacterium smegmatis*. *Bioorganic & medicinal chemistry*, Vol.12, No.24, (December 2004), pp. 6397-6413, ISSN 0968-0896
- Washko, M.E., & Rice, E.W. (1961) Determination of glucose by an improved enzymatic procedure. *Clinical chemistry*, Vol.7, (October 1961), pp. 542-545, ISSN 0009-9147

- WHO (2010) Global tuberculosis control report 2010. Summary. *Central European journal of public health*, Vol.18, No.4, (December 2010), pp. 237, ISSN 1210-7778
- Wiker, H.G., & Harboe, M. (1992) The antigen 85 complex: a major secretion product of *Mycobacterium tuberculosis*. *Microbiological reviews*, Vol.56, No.4, (December 1992), pp. 648-661, ISSN 0146-0749
- Wilming, M., & Johnsson, K. (1999) Spontaneous Formation of the Bioactive Form of the Tuberculosis Drug Isoniazid. *Angewandte Chemie (International ed. in English)*, Vol.38, No.17, (September 1999), pp. 2588-2590, ISSN 1521-3773
- Woodruff, P.J.;Carlson, B.L.;Siridechadilok, B.;Pratt, M.R.;Senaratne, R.H.;Mougous, J.D.;Riley, L.W.;Williams, S.J., & Bertozzi, C.R. (2004) Trehalose is required for growth of *Mycobacterium smegmatis*. *The Journal of biological chemistry*, Vol.279, No.28, (July 2004), pp. 28835-28843, ISSN 0021-9258
- Zhang, J.H.;Chung, T.D., & Oldenburg, K.R. (1999) A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *Journal of biomolecular screening : the official journal of the Society for Biomolecular Screening*, Vol.4, No.2, (April 1999), pp. 67-73, ISSN 1552-454X

IntechOpen



Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance

Edited by Dr. Pere-Joan Cardona

ISBN 978-953-307-948-6

Hard cover, 376 pages

Publisher InTech

Published online 15, February, 2012

Published in print edition February, 2012

In 1957, a *Streptomyces* strain, the ME/83 (*S.mediterranei*), was isolated in the Lepetit Research Laboratories from a soil sample collected at a pine arboretum near Saint Raphael, France. This drug was the base for the chemotherapy with Streptomycin. The euphoria generated by the success of this regimen led to the idea that TB eradication would be possible by the year 2000. Thus, any further drug development against TB was stopped. Unfortunately, the lack of an accurate administration of these drugs originated the irruption of the drug resistance in *Mycobacterium tuberculosis*. Once the global emergency was declared in 1993, seeking out new drugs became urgent. In this book, diverse authors focus on the development and the activity of the new drug families.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ayssar A. Elamin, Matthias Stehr and Mahavir Singh (2012). The Cord Factor: Structure, Biosynthesis and Application in Drug Research – Achilles Heel of *Mycobacterium tuberculosis*?, *Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance*, Dr. Pere-Joan Cardona (Ed.), ISBN: 978-953-307-948-6, InTech, Available from: <http://www.intechopen.com/books/understanding-tuberculosis-new-approaches-to-fighting-against-drug-resistance/the-cord-factor-structure-biosynthesis-and-application-in-drug-research-achilles-heel-of-mycobacteri>

INTech
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen