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Analyzing Different Acetyl Co-A Metabolizing Enzymes as Potential Drug Targets against *Mycobacterium tuberculosis*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mycobacterium tuberculosis, the causative agent of tuberculosis, is responsible for the deaths of million people around the globe. The scenario is worse than ever due to the emergence of drug resistant strains which are widely spread throughout the globe in much faster ways. To control this worst situation, we need to speed up the search for novel drugs which can specifically kill drug resistant bacteria in collaborative ways amongst academic, clinician and industry. Among different metabolic pathways, fatty acid synthesis pathway has always been a very attractive area for the drug target because of its crucial role during the infection and further in long term survival of pathogen inside the human host. In this review article, we analyzed the role of important and crucial enzymes, which are responsible for the influx and efflux of acetyl co-A substrate, a central hub of metabolites, as potential drug targets against *M. tuberculosis*.

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1. INTRODUCTION

Mycobacterium tuberculosis is one of the most successful pathogens of humankind, which is responsible for causing Tuberculosis (TB) [1]. TB is one of the leading infectious disease that leads to mortality, mainly in economically poor countries of Africa and Asia [2]. As reported, TB causes 5 million new cases with 2 million deaths annually around the globe and 1/3rd (~2 billion) of the world population are infected with dormant TB. This dormant form TB can become active TB anytime once the Immune system of the host is compromised as in HIV, diabetes, organ transplant or older age peoples. TB is a leading cause of death in HIV positive persons. The death rate of HIV positive patients with TB is 10 % annually as compared with 10 % throughout the life span in case of healthy person having latent TB [3]. Because of this burden and severity of disease, WHO has declared TB as global health disease in the year 2003 and 24th March is observed as World TB Day [4]. Every year, this day is taken as an opportunity to raise awareness, the status and control efforts about the burden of tuberculosis worldwide. On World TB Day 2015, WHO calls on governments, affected communities, civil society organizations, health-care providers, and international partners to join the drive to roll out this strategy and to reach, treat and cure all those who are ill today [5].

It is now possible to control TB if both the diagnosis and treatment start on time. There are 13 different types of drugs available in the market for the treatment of different form of TB. According to new guidelines, standard regimen for the new TB patients includes initially a combination of Rifampicin (R), Isoniazid (H), Ethambutol (E) and Pyrazinamide (Z) for two months and then R+H for the next four months, which is commonly prescribed as 2HRZE/ 4HR under DOTS (Direct Oral Therapy Short course) as recommended by WHO [6]. This long duration of TB treatment and its side effects motivates patients to discontinue the course in-between which leads to the outcome of Multi/eXtremely drug resistant MDR/XDR forms of TB, which is difficult to treat with the existing front line drugs [7]. The MDR TB patients are then treated with second line drugs (Amikacin, Kanamycin and Capreomycin) but the cost of second line drugs is ~20 times more than the existing front line drugs [8]. The severity continues when the MDR become XDR where the third line of drugs fluoroquinolone (either OFX, LVX, or MXF) with Ethionamide, Pyrazinamide, and Amikacin) are enforced which are not only costly (~200 times) but have severe side effects along with social discrimination that leads to high frustration in TB patients [9]. In most shocking observation, there are already 12 reports of Total Drug Resistant (TDR) patients where TB is un-treatable with existing drugs are found since 2012 in India [10].

There is an urgent requirement of some novel drugs which can kill the bacilli of any form inside the human host without any side effects and no-cross reactivity with other HIV, cancer, diabetics or Immunosuppressed drugs. Furthermore, the new drugs can shorten the existing TB drug course from 6-8 months to 3-4 weeks. So far, not a single new antibiotic has come into market after Rifampicin in 1970s, that could meet the above mentioned requirement of ideal TB drugs [11]. It's a high time that we speed up the drug discovery process where experts from Academia, Research and Industry can come together for this social cause. Their initial ideas can be proved through the high end techniques like microarray studies, gene expression studies, new molecule synthesis, enzyme-molecule docking and screening of potential molecules to get some novel hits through initial research work [12]. Further the clinical trial of potent molecules under the guidance of Industrial collaboration is needed to find out some novel molecules to fight against this deadly disease.

There are several review articles available on drug discoveries against tuberculosis where authors reviewed the reported data for analyzing the potential enzymes as drug targets, among which enzymes involved in fatty acid metabolic pathway have always been an attractive target followed by nitrogen, carbon and nucleic acids metabolic pathways [13]. If we analyze the whole genome sequence of *M. tb*, 15% of functional genes are responsible for the fatty acid metabolism, which indirectly indicates that fatty acid metabolic pathways are crucial for the growth and survival of *M. tb*. This could be because of the alternate source of carbon and energy generated in adverse condition inside the human macrophage by glyxolate and fatty acid oxidation pathways [14]. Glyxolate pathway

fulfills the carbon requirement by gluconeogenesis reaction and utilized excess acetyl co-A (Ac Co-A) generated from ß-oxidation pathways to form oxaloacetate, which convert into phenol-enol pyruvate and enter in the gluconeogenesis pathway for glucose formation. Furthermore, nitrate, nitrite and ammonia assimilation pathways of nitrogen metabolism suggested fertile area for potential drug discovery against TB [15]. This could be because of firstly dependency on nitrate and nitrite metabolisms for maintaining redox potential in the absence of oxygen and secondly absence of nitrate and nitrite pathways in human offers the extra advantage to target pathogen [16]. Although, the carbon and nucleic acid metabolism need to be more extensive review as a drug discovery point of view.

Hence, we need to approach from multiple ways, including some existing and novel ways for killing this deadly bug. In one such attempt, here we are analyzing the role of enzymes responsible for the efflux and influx of Ac Co-A as a potential drug target against *M. tb*. Ac Co-A is positioned at the metabolic crossroads of glycolysis, fatty acid oxidation, ketogenesis, amino acid metabolism, the TCA cycle, and lipid synthesis, suggesting that Ac Co-A may be an ideal metabolite for the cells to sense their nutrient status. Hence, Ac Co-A serves as a central hub of substrate whose concentration decides the activation of several anabolic or catabolic pathways of bio-molecules directly or indirectly along with coordination of other signal factors like NADH/$FADH_2$/ATP, glucose, citrate, hormones, oxygen inside the cell.

1.1 Acetyl Co-A: A Central Hub of Cellular Metabolites

Acetyl Co-A, a 2-carbon sulfhydryl molecule whose main function is to convey the carbon atoms within the acetyl group into the citric acid cycle (Krebs cycle) which will be oxidized further for energy production. It can directly associate or attached with other acetate or carbonate ions present in the molecule like CO_2 with the help of ATP to form high energy, carbon molecule (activated molecule) which can be a substrate for fatty acid synthesis [17]. The acetyl group of Ac Co-A is linked by a "high energy" thioester to the sulfhydryl portion of the β-mercaptoethylamine. It is this thioester bond that makes Ac co-A one of the "high energy" compounds. Hydrolysis of the thioester bond is highly exergonic (-31.5 kJ). Here we are analyzing the enzymes responsible for efflux (production) and influx (utilization) of Ac Co-A of different metabolic pathways as a potential drug target against *Mycobacterium tuberculosis*.

1.1.1 Ac Co-A production

Ac Co-A is produced either directly as newly synthesized molecule from acetate or through various degradation process of molecules like glucose, fatty acids and amino acids which can act as a source of energy production, reducing substrates etc (Fig. 1).

1.2 De-novo Synthesis of Ac Co-A

Adenosine monophosphate (AMP)-forming acetyl Co-A (Ac Co-A) synthetase (Acs, EC 6.2.1.1) is a key enzyme for conversion of acetate into Ac Co-A. The enzyme is a member of the acyl-adenylate forming enzyme superfamily, and is widespread in all three domains of life. In bacteria, Acs is the preferred route of acetate assimilation, when the concentration of acetate in the environment is low (≤10 mM). In eukaryotes, Acs is the only route for the activation of acetate into Ac Co-A [18].

This enzyme undergoes a two-step reaction. In the first-half reaction, Acs combines acetate with ATP to form acetyl–adenylate (Ac-AMP) intermediate (Equation 1). In the second-half reaction, it can then transfer the acetyl group from Ac-AMP to the sulfhydryl group of Co-A, forming the product Ac Co-A (Equation 2)

$$\mathrm{E} + \mathrm{acetate} + \mathrm{ATP} \leftrightarrow \mathrm{EAcAMP} + \mathrm{PPi}$$

$$\mathrm{EAcAMP} + \mathrm{HSCoA} \leftrightarrow \mathrm{E} + \mathrm{AcCoA} + \mathrm{AMP}$$

Several crystal data of Ac Co-A synthetase from both yeast and prokaryotes are available which showed a distinct structural difference which can be used to specifically target the bacterial enzyme. Although mycobacterial Ac Co-A synthetase structure is not resolved so far which could be more helpful in designing specific drug molecule that could kill pathogens. Although Allicin, a natural antibiotic from onion found to be a potent inhibitor of both prokaryotic and

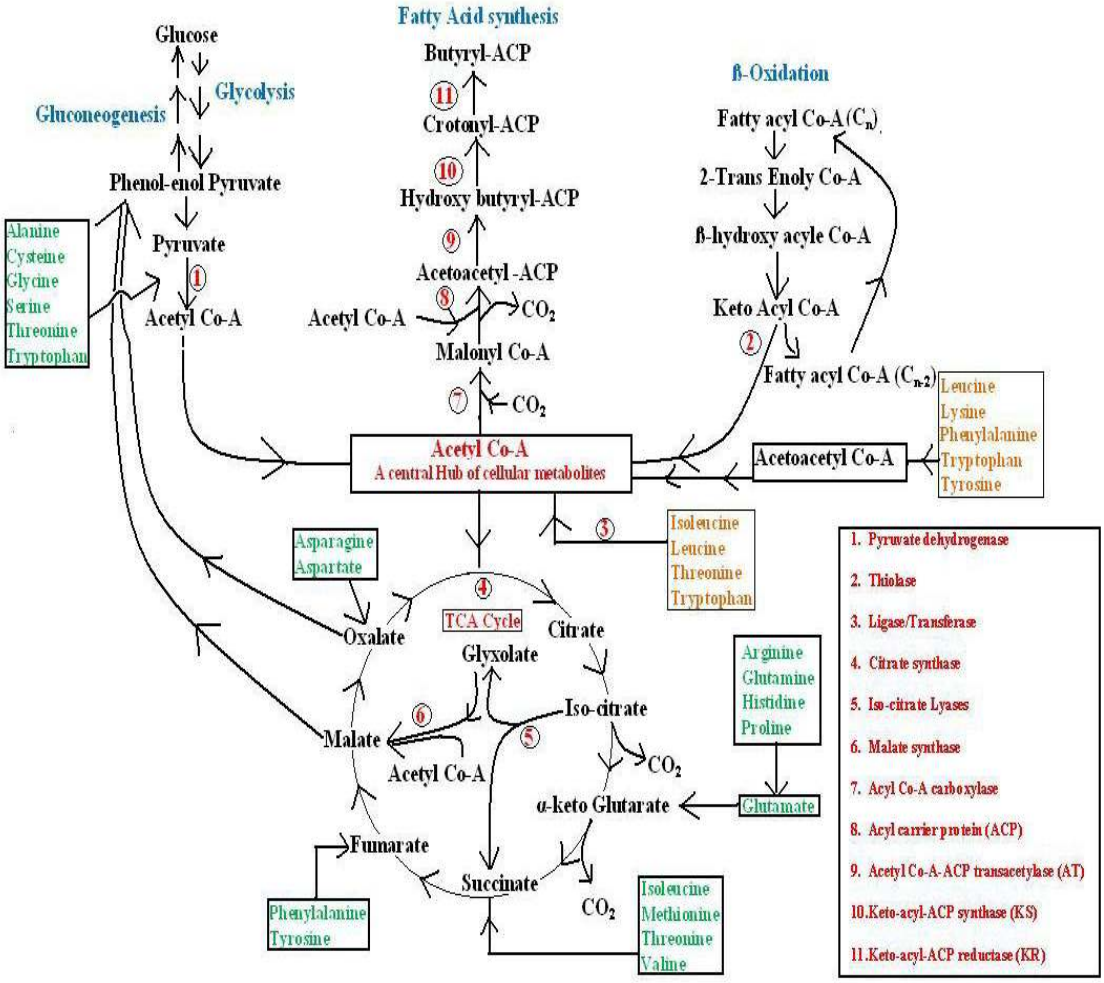

Fig. 1. Circuit diagram of Acetyl Co-A metabolizing pathways. Specific numbers mentioned in several pathways shows the enzyme involved in conversion of substrate into product and could be the potential drug target against *Mycobacterium tuberculosis*

eukaryotic Ac Co-A synthetase by binding to the enzyme in a non-covalent and reversible manner with an I50-value < 10 µM [19]. This provides an additional rich area which can be explored for the drug discovery against *Mycobacterium tuberculosis*.

2. GLYCOLYSIS

The glucose molecule undergoes into the glycolysis process in the cytoplasm of cells leads to the formation of pyruvate along with ATP and NADH. The reducing NADH molecule enters into the electron transport chain in ETC complex-I and oxidized further to form ATP, which is a direct source of energy [20]. Pyruvate further converts into Ac Co-A by an enzyme pyruvate dehydrogenase complex (PDK). This enzyme is multimeric in case of bacteria and important point of regulation by phosphorylation/ dephosphorylation by pyruvate dehydrogenase kinase, which gets activated or suppressed by the concentration of ATP, NADH, $FADH_2$, Ac Co-A and pyruvate. The pyruvate dehydrogenase complex is made up of three subunits namely pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2) and dihydrolipoyl dehydrogenase (E3).

Initially, pyruvate get attached with the TPP factor of E1 which make the release of carboxyl group from pyruvate and attach hemiacetal group to TPP which is further form thioester bond with a sulphohydroyl group of Co-A-SH by lipoyl group of E2 subunit. Further, lipoyl group

is reduced by NADH with the release of Ac Co-A [21].

The pyruvate dehydrogenase (E1) and dihydrolipoyl transacetylase (E2) of PDH complex are products of *aceE* and *dlaT* gene, while dihydrolipoyl dehydrogenase (E3) is coded by the *lpdC* gene of *M. tb* [22]. The *dlaT* and *lpdC* genes are also responsible for the coding of branched chain keto acid dehydrogenase (BCKAD) and PNR/P enzyme complex respectively. The BCKAD enzyme plays a crucial role in the degradation of branched chain amino acids while PNR/P is known to be important for detoxifying the peroxinitrite (RNI) released by activating human host macrophages to kill intracellular *M. tb* [22]. It has been shown that deletion of *dlaT* gene had a pronounced effect on *M. tb* growth in standard medium in vitro, sensitive to RNI and attenuates *M. tb* in mouse. Also, specific inhibitor of *M. tb* DlaT enzyme showed selective killing of non-replicating dormant bacilli. Furthermore, in recent studies showed that mutant of *lpdC* gene in *M. tb* was far more attenuated as compared to the *dlaT* mutant in mice, which indicate that both *dlaT* and *lpdC* genes can be potential drug target against *M. tb*. The triazospirodi-methoxybezoyl, an inhibitor of bacterial LpdC will specifically kill the *M. tb* sparing human LpdC. Also, these *M. tb* genes offer advantage of potential drug target due to structure differences with human DlaT and LpdC complex [23]. Furthermore, a more recent, N-methylpyridine 3-sulfonamides as potent and species-selective inhibitors of *M. tb* Lpd affording >1000-fold selectivity versus the human homolog was identified. This inhibitor is having high potency and selectivity due to its binding site is the lipoamide-binding site of *M. tb* Lpd, a site different from the NAD^+/NADH pocket targeted as previously triazaspirodimeth-oxybenzoyl inhibitors [24].

Although, E2 and E3 subunits of pyruvate dehydrogenase complex are suggested as a potential drug target while the potential role of the E1 subunit as drug target has not been done so far. Although, studies showed that the E1 subunit is known to be essential and mutant of E1 subunit is slow in survival rate as compared to wild type in *E .coli* [25]. Crystal structure of the E1 subunit from *E. coli* is available although no such structure studies have been carried out in *M. tb* [26]. The potential role of the E1 subunit as drug target can be further explored by studying the effect of survival of E1 mutant on *M. tb* to know its essentiality and then further structure-molecule docking studies can be done for screening of inhibitors.

3. CHOLESTEROL METABOLISM

Pulmonary Tb mainly resides in the alveolar macrophages induces host immune response to form the granuloma, an inflammatory lesion composed of lymphocytes, macrophages, and multinucleated giant cells [27]. TB granuloma has an increased abundance of lipid and cholesterol molecules due to imbalance between the export of low-density lipoprotein (LDL) through macrophage and the excess uptake of LDL which lead foamy macrophages [27]. Furthermore, gene expression studies done on in- vivo residing *M. tb* reveals that induced expression of ~200 different genes which are involved in either its transportation inside the granuloma structure or cholesterol catabolism and which help in its long term survival and pathogenesis.

The transportation of cholesterol inside the granuloma is mainly carried by ABC- like ATP dependent manner *mec4* locus further its deletion inhibits the growth of bacilli on cholesterol, but not on glycerol [28]. Furthermore, mutant *mec4 M. tb* was unable to grow in INF-γ activated macrophages and this mutant was able to initially persist in lung infected C57BL/6 mouse, but its CFU declines significantly after 4 weeks of infection. These all indicate that survival of bacilli on cholesterol during the chronic phase of its infection.

Cholesterol structure is made of four sterol ring A, B, C and D with attached long chain fatty acids. Here we are discussing its catabolic process in step wise.

3.1 A & B Ring Catabolism

A and B rings degradation of cholesterol required total of 10 enzymes with subsequent 8 enzymatic reactions which are ClassI of oxidoreductase type of enzymes. The first reaction of ring metabolism is the oxidation and isomerization of cholesterol to form cholest-4-ene-3-one. In bacteria, this reaction is catalyzed either by a 3β-hydroxysteroid dehydrogenase (3β-HSD) or cholesterol oxidase (ChOX). The genome of *M. tb* contains designated gene for 3β-HSD and ChoX which is *Rv1106c* and *Rv3409c* respectively. Recombinant 3β-HSD proteins oxidized the cholesterol as substrate

into cholest-4-ene-3-one while mutant of *Rv1106c M. tb* strain unable to oxidize the cholesterol substrate. Although, *choX* mutant studies showed non-dependency of this gene for catabolizing cholesterol, but plays an important role in virulence of bacteria as *M. tb* as Δ*choX* strain showed attenuated virulence in both peritoneal macrophages and mouse models of infection [29]. Moreover, a *3*β-*hsd* mutant strain replicated at a similar rate to wild type in macrophages, and infection studies in the guinea pig infection model showed identical CFU in the lungs of wild type, *3*β-*hsd* mutant, and *3*β-*hsd* complement. It was concluded that *3*β-*hsd* is not necessary for nutrition acquisition, likely because during infection *M. tb* has access to and utilizes multiple carbon sources [30].

The second step of cholesterol A & B ring metabolism is 1, 2-desaturation of cholest-4-ene-3-one to a di-α,β enone product which is catalyzed by 3-ketosteroid-Δ1-dehydrogenase (KstD). In *M. tb,* KstD is encoded by *Rv3537* which is functionally active gene as proved by its enzyme activity and mutant studies. KstD is required for growth of *M. tuberculosis* in minimal medium supplemented with cholesterol [31]. In resting THP-1 macrophages, growth of a Δ*kstD* knockout is attenuated compared to H37Rv wild-type strain.

Next in ring catabolism, a 3-ketosteroid-9α-hydroxylase catalyzes the addition of a hydroxyl group at C9, which leads to the subsequent aromatization of the A ring and opening of ring B. This enzyme is a two-component Rieske monooxygenase made up of KshA, the oxygenase component, and KshB, the reductase component. The 3-ketosteroid-9α-hydroxylase homologs in *M. tb*, KshA (*Rv3526*) and KshB (*Rv3571*) have been recombinantly expressed in *E. coli* and purified. Activity was reconstituted *in vitro* with several substrates including AD, ADD, the Co-A thioester of 3-oxo-4-pregnene-20-carboxylic acid, and the Co-A thioester of 3-oxo-1,4-pregnadiene-20-carboxylic acid. The *M. tb* Δ*kshA*/Δ*kshB* double mutant was unable to grow on cholesterol, AD, or 5α-androstane-3,17-dione [32]. It was also shown that the deletion of either *kshA* or *kshB* resulted in the rapid clearance of infection in an *in vitro* macrophage model, or an *in vivo* mouse infection model. In a mutant where only *kshA* was deleted there was no observed phenotypic change.

Next is conversion of 3-hydroxy-9,10-secoandrost-1,3,5(10)- triene-9,17-dione (3-HAS) to HIP and 2-hydroxy-hexa-2,4-dienoic acid (HHD) via three intermediate products which are 3,4-dihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione (3,4-DSHA), 4,5-9,10-diseco- α3-hydroxy-5,9,17-trioxoandrosta-1(10),2-diene-4-oic acid (4,9-DSHA). These enzymatic conversions carried by HsaA & HsaB, HsaC and HsaD respectively which are present in *M. tb*. These conversions have been demonstrated *in vitro* with recombinant HsaA/B, HsaC and HsaD [33]. The crystal structure of HsaA demonstrated that there was an elongation of the substrate tunnel at C17, and this information in conjunction with the relatively weak substrate specificity constants of complete side chain degraded substrates suggests that *in vivo* partially degraded side chain substrates are utilized as well [30]. Knockout studies of *hsaA* (*Rv3570c*), *hsaB*, *hsaC* and *hsaD* separately reveled that all these genes are essential and required for growth of *M. tb* in macrophages and showed attenuated growth in immune-compromised mice and guinea pigs [34].

In *M. tb,* HsaEFG (*Rv3534c*/*Rv3535c*/*Rv3536c*) are hypothesized to metabolize HDD. HsaF and HsaG were shown to form a heterotetrameric complex, formation of which is required for activity [35]. Aldolase HsaF catalyzes the cleavage of 4-hydroxy-2-oxohexanoate to propionaldehyde and pyruvate in the presence of NAD^+ and Co-A. Volatile propionaldehyde is channeled to ehydrogenase HsaG where is it converted to propionyl Co-A. The activity of HsaE has not been verified. Selection studies reveal that *hsaE, hsaF* and *hsaG* are not required for growth on cholesterol, although their deletion does result in slower growth on cholesterol as a carbon source. Because a *hsaE, hsaF,* or *hsaG* mutant still has the requisite genes required for catabolism of the side chain and the C & D rings of cholesterol, growth remains possible in minimal medium supplemented with cholesterol. The reason for slower growth could be due to toxicity of accumulated metabolites, or other secondary effects.

All these studies suggested that the enzymes involved in catabolizing the A & B ring of cholesterol are crucial for the survival of M. tb when cholesterol is available in-vivo as carbon source. Although detailed studies related to its structure-molecule correlation and further screening of inhibitors against these enzymes are not actively carried out so far.

3.2 Steroid C & D Ring Degradation

How extensively the C & D rings are degraded by M. tb is not known. After initial catabolism of the A & B rings, it is presumed that the C & D rings are catabolized in an oxidative manner and acetyl Co-A and/or propionyl Co-A are generated. This assumption is supported by the fact that, after a significant lag phase, M. tb is able to grow on (3aα-H-4α(3′-propanoyl-CoA)-7aβ-methylhexa-hydroindane-1,5-dione) HIP, a C & D ring derivative, as a sole carbon source, thus highlighting the fate of at least some of the C & D ring carbons. The precise fate of HIP in M. tb has not yet been established. The degradation of the hexahydroindanone C & D steroid ring intermediate begins through the thio-esterification of the propionate moiety left over from A & B ring oxidation. FadD3, an acyl Co-A ligase, was recently demonstrated to perform this function on HIP [36]. The M. tb fadD3 (Rv3561) gene was knocked out and comparative studies with control was done which indicated no significant change in both the form. Steady-state kinetic analysis of recombinantly expressed FadD3 from M. tb demonstrated that the specificity constant for HIP, which contains a keto group at the 5 position of the indanone, is 165 times greater than the specificity for 5α-OH HIP, where this is a hydroxyl moiety.

Till now, it's not known exactly which specific enzymes are responsible for the degradation of complete C and D ring of cholesterol, but in proteobacteria Comamonas. testosteroni TA441, a gene disrupted mutant of ORF 18 which supposed to degrade testosterone, was grown on ADD, chenodeoxycholic acid, and cholic [37]. This mutant studies showed the accumulation of HIP when grown on ADD which indicate that this gene could be a probable Co-A ligase essential for further degradation of the C & D ring steroid nucleus. Additional substitutions like hydroxyl groups on the steroid ring system, as seen for cholic acid, for example, did not preclude metabolism to the step encoded by ORF18. However, these kinds of biochemical characterization and its further confirmation are not reported so far from M. tb which again offers the possibilities for research which can fill this lacking information in M. tb. This can be done by initially homology sequence analysis in M. tb genome with already annotated genes from organisms and then mutant studies of those genes with further its phenotypic characterization.

4. FATTY ACID DEGRADATION

Ac Co-A is formed majorly from fatty acid degradation pathway by breaking the carbon bond at ß-position, hence known as ß-oxidation pathway [38]. This pathway required four steps which include dehydrogenation by fatty acyl Co-A dehydrogenase followed by addition of water molecule by enzyme enoyl Co-A hydrates. Further, the hydrogenated molecule was dehydrogenated again, but hydrogen acceptor is NADH rather than FADH2 as in the first step of double bond formation. The last step is breakage of C-C bond and the formation of new thio-ester bond with coenzyme-A by enzyme thiolase with the release of Ac Co-A and Cn-2 fatty acyl Co-A as a product which become a substrate for the next cycle. The last step of ß-oxidation is rate limiting steps and is regulated by several factors like concentration of ATP, ADP, Ac Co-A, fatty acid, NADH, FADH2, FAD+, NAD+, Co-A etc. Bioinformatics analysis reveals that eight and thirteen different types of thiolase genes are present, including one and two thiolase like proteins (TLPs) in M. tb and M. smegmatis genome respectively [39,40]. The crystal structure of M. tb trifunctional fatty acid ß-oxidation complex is available which indicates the presence of different fold for the accommodation of bulkier Fatty acyl chains. Recently, a different thiolase from M. tb, FadA5 structure has been solved, which is catalyzing the last step of the β-oxidation reaction of the cholesterol side-chain degradation. This was shown to be of importance during the chronic stage of TB infections. The steroid-bound structure provides a solid basis for the development of inhibitors against FadA5. So far, no mutant studies related to thiolase has been attempted in M. tb although thiolase minus Pseudomonas strain was not able to survive in growth medium which strongly suggest its important role [41]. 4-bromooctanoic acid acts as an irreversible inhibitor of thiolase and blocks the ß-oxidation completely and helping out treating insulin-dependent diabetes mellitus and stable angina pectoris [42]. All these information indicates that fatty acid degradation is important pathways in which thiolase could be crucial for the survival of M. tb under different conditions. It is yet to explore the dependency of M. tb on specific thiolase and hence its role as potential drug candidates is yet to be analyzed. Although this can be confirmed by the knockout of different genes and further analyze its physiological effect on the survival of M. tb. Once the specific gene's role in the bacterial

survival is known then its recombinant and structure-molecule docking studies can be done.

5. AMINO ACIDS DEGRADATION

The pathways of amino acid catabolism, taken together, normally account for only 10 % to 15 % of the human body's energy production; these pathways are not nearly as active as glycolysis and fatty acid oxidation [43]. Flux through these catabolic routes also varies greatly, depending on the balance between the requirements for biosynthetic processes and the availability of a particular amino acid. The 20 catabolic pathways converge to form only six major products, all of which enter the citric acid cycle. From here the carbon skeletons are diverted to gluconeogenesis or ketogenesis or are completely oxidized to CO_2 and H_2O. All or part of the carbon skeleton of seven amino acids are ultimately broken down into acetyl Co-A. Five amino acids are converted to α-ketoglutarate, four to succinyl Co-A, two to fumarate, and two to oxaloacetate (OAA). Parts or all of six amino acids are converted to pyruvate, which can be converted to either acetyl Co-A or oxaloacetate. Most of the enzymes which are responsible for the removal of carbon skeleton from amino acids in the form of aceto-Ac Co-A or Ac Co-A, are done by ketoacyl Co-A transferase which is subfamily of transferase enzyme [44]. The enzymes of transferase group are known to be the potential drug targets like the β-ketoacyl-ACP synthases (fadH, kasA and kasB) which is distinct and plays a vital role in several cellular processes including pathogenesis [45].

Another enzyme which is keto-butyrate-Co-A ligase involves in transferring the acetate group from the carbon backbone of amino acid into Co-A subunit with the activation through Adenyl TriPhosphate (ATP). Adenylation or adenylate-forming enzymes (AEs) are widely found in nature while in *Mycobacterium tuberculosis* encodes for more than 60 adenylating enzymes, many of which represent potential drug targets due to their confirmed essentiality or requirement for virulence. Several strategies have been used to develop potent and selective AE inhibitors including high throughput (HTS) screening, fragment-based screening, and the rational design of bisubstrate inhibitors that mimic the acyladenylate [46]. Some of the these AEs play an important role in aminoacyl t-RNA synthetases (aaRSs), MenE required for menaquinone synthesis, the FadD family of enzymes including the fatty acyl-AMP ligases (FAAL) and the fatty acyl Co-A ligases (FACLs) involved in lipid metabolism. Most of the transferase or ligase play an important role in the metabolic pathway whose dependency for the survival of bacilli can be the fertile area of drug discovery.

5.1 Ac Co-A Utilization

Ac Co-A, a high energy substrate is utilized for the formation of several important bio-molecules like, glucose, fatty acid synthesis, amino acid formation which play an important role in the overall growth and division of cells.

5.2 Citrate Synthases

The 1st enzyme of the Krebs cycle, citrate synthase, which is responsible for merging the 2-carbon Ac Co-A into Oxaloacetate (4-C) to form 6-C citrate, is known to be up-regulate its gene expression during hypoxia condition inside the human macrophage. Three genes are annotated as citrate synthases, (cit3). Cit3 shows significant up-regulation and could be an attractive target if the other two enzymes do not provide a functional alternative. Also the citrate synthase activity in lysate of M. tb shown to ~400 nM/min/mg of protein, which is 2- and 4-fold higher than M. smegmatis and M. bovis BCG under similar condition [47]. This up-regulation of its gene expression and higher enzyme activity clearly indicates that citrate synthase enzyme having the supportive role in the survival of bacteria in a dormant condition. Although, survival studies of citrate synthase mutant form of *M. tb* is not yet done under different growth conditions while citrate synthase minus *E. coli* is unable to grow on LB medium and LB medium with acetate [48]. Furthermore, crystal structure of citrate synthase from *M. tb* has been solved this year only which showed that it consists of a dimer with a typical prevalent a-structure with N-terminus of each monomer is characterized by a large unstructured region with the exception of the presence of two small, antiparallel b-sheets. At the interface area of the two monomers are located eight helices (four for each monomer) which share a contact area of approximately 3700 A˚. The overall electron density presents two large gaps between residues 290–314 and 349–365 in correspondence of the Ac Co-A binding site that, in the absence of the cofactor, is highly flexible and unstructured. Comparison of the arrangement of this molecule with other OAA molecules of homologous citrate synthases-OAA

binary structures deposited in the protein databank (PDB) showed for M. tb citrate synthase a different arrangement of the OAA molecule. This observed difference is because of new interaction of Arg391 and Asp235 and losing hydrogen bonding with His232 and His280 residues. During catalysis, citrate synthase oscillates between an "open" and a "closed" conformation: the "open" conformation allows binding of OAA which then directs the sealing of the OAA, binding site and generates the binding site accommodating Ac Co-A [49]. Although, the inhibitor screening against citrate syntheses enzyme are not yet started.

5.3 Glyxolate Shunt Pathway

The TCA cycle is a major source of energy which complete only in the presence of oxygen. The oxygen tension is low inside the granuloma structure leads to halt of the TCA cycle. This hypoxic condition leads to activation of glyxolate pathways which convert citrate into glyxolate and succinate by isocitrate lyases (ICL). Further glyxolate combines with Ac Co-A to form malate by enzyme malate synthase (MS) [50]. Thus glyxolate pathway shunts the TCA cycle and saves two carbon releasing steps in form of CO2 and replacing TCA cycle as anaplerotic way. Now, both malate and oxalate (formed from succinate) are converted into phenol-pyruvate to enter into a Gluconeogenesis pathway to form glucose. It has been shown that ICL and MS were up-regulated during hypoxic condition inside the human macrophages. While, icl deficient M. tb does not have much effect on its initial survival, but attenuates during the later stage of persistence in the mice model system when compared with wild type *M. tb*. Also, the absence of these genes in human makes them an attractive target against TB drug designing program. Since the crystal structure of both ICL and MS are known, enzyme molecule docking studies along with enzyme substrate/inhibitor binding studies can be useful for identification of lead molecules/inhibitors [51]. Recently a lot of progress has been made relating to isocitrate lyase inhibitors, overviews structural analogues of several metabolic intermediates (3-nitropropionate, 3-bromopyruvate, itaconate, itaconic anhydride), peptide inhibitors, and recently developed inhibitors with various chemical structures. The largest inhibitory activity against isocitrate lyase (IC(50) of 0.10 ± 0.01 μM) and concomitantly a significant antimycobacterial activity has been presented by fluoroquinolone derivative 1-cyclopropyl-7-[3,5-dimethyl-4-(3-nitropropanoyl) piperazin-1-yl]-6-fluoro-8-methoxy-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid, which has incorporated 3-nitropropionyl group as one of the structural analogue of succinate, a metabolic intermediate [52]. So far these mentioned molecules are excellent inhibitor but are toxic to the human host which limits their potential as drug candidates. Still there are scopes of making these molecules non-toxic and further screening inhibitors from both natural and/ or synthesis origin can be rewarding (Table 1).

5.4 Fatty Acid Synthesis

The unusual mycolic acid rich cell wall structure along with several other components play important and crucial role for both viability and pathogenicity of M. tuberculosis [53]. This impermeable barrier is enough to provide the resistance against both hostile environments and antibiotics used to kill bacilli along with ability to enhance the host immune response. The mycolic acids present in cell wall are produced from elongated fatty acids and it is this lipid layer that has proven most interesting from a drug target point of view. So far, the front line anti tubercular drugs such as Isoniazid, Ethambutol, Pyrazinamide primarily targets cell wall synthesis, including mycolic acid synthesis (Table 2) [54]. Thus, the unique structure of this cell envelope and the importance of its integrity for the viability of the organism suggest that the search for novel drug targets within the array of enzymes responsible for its construction may still prove fruitful. And that's the reason that fatty acid synthesis is most attractive pathway where most of the enzymes involves either in activation of Ac Co-A to form malonyl Co-A by fatty acyl Co-A carboxylase (ACCases) and further elongation of fatty acyl Co-A chain by fatty acid synthase complex are potential drug target against *M. tb* [55]. These enzymes are not only targeting against mycobacterial infection, but also against other human diseases like cancer, diabetes, obesity, and bacterial infection.

Among the potentially attractive drug targets are the enzymes that provides the building blocks for lipid biosynthesis, the acyl Co-A carboxylases (ACCases). These enzymes catalyze the biotin-dependent α-carboxylation of acetyl- and/or propionyl Co-A to generate malonyl- and methyl malonyl-Co-A, respectively. In mycobacteria, these metabolites are used by the fatty acid synthase I (FAS I) for the biosynthesis of membrane fatty acids, as well as by the FAS II

and the polyketide synthases for the biosynthesis of the complex lipids present in the cell wall, such as the long-chain α-alkyl, β-hydroxymycolic acids, the phthiocerol dimycocerosates and sulfolipids. The bacterial ACCases could be suitable targets for antibacterial drug discovery, and its inhibitor with antibacterial activity, derived from pyrrolidine dione natural products, was characterized and proposed as a group of promising antibacterial compounds with a novel mode of action very recently [56]. The successful determination of the crystal structure of AccD5 of M. tuberculosis allowed us to carry out an extensive in silico screening of several compound databases that resulted in the identification of a number of putative ACCase inhibitors [57].

Fatty acid synthase is a complex multimeric protein consists of seven different polypeptides with three other proteins involved in some stages of the fatty acid synthesis process. These seven polypeptides are Acyl carrier protein (ACP), Ac Co-A-ACP transacetylase (AT), Keto-acyl-ACP synthase (KS), Malonyl Co-A ACP-transferase (MT), Keto-acyl-ACP reductase (KR), hydroxy acyl-ACP dehydratase (HD) and enoyl-ACP reductase (ER). Initially, malonyl Co-A formed from Ac Co-A and CO2 by ACC, attached on sulfhydryl group of ACP while next Ac Co-A linked at KS by AT. Further, condensation reaction occurs on ACP protein followed by dehydration and reduction where the hydrogen donor or accepter is NADPH molecule. Once, the fatty acyl Co-A chain increased by 2-carbon chain, complete complex moves again to KS while ACP becomes free to accept next malonyl Co-A.

Here, each of seven polypeptides of fatty acid synthase complex can be a potential drug target against *M. tb.* The ACP protein, which is a small protein (Mr 8860) contains prosthetic group 4'phosphopantetheine. This prosthetic group acts as flexible arm which carry the fatty acyl Co-A complex from one subunit to the other subunit of FS complex. This interaction of ACP and fatty acyl Co-A is on the surface of proteins due to the presence of oppositely charged amino acids domains. Designing of any inhibitor, which can block the binding between ACP and any of the other six subunit of fatty acid synthase complex, could obstruct the fatty acid synthesis pathway [58]. The covalent attachment of the 4'-phosphopantetheine moiety of Coenzyme A is catalyzed by phosphopantetheinyl transferases (PPTases) enzyme. PptT, one of the two PPTases produced by mycobacteria, is involved in post-translational modification of various type-I polyketide synthases required for the

Table 1. Acetyl Co-A metabolizing enzymes as potential drug targets against *M. tuberculosis.*

Sl. no.	Pathway	Drug target	Functionally characterized	Structure known	Inhibitors	Potential drug
1	Acetyl Co-A de-novo synthesis	Ac Co-A synthetase	Yes	Not done	Not done	Not done
2	Glycolysis	PDC- E1	Yes	Not done	Not done	Not done
		E2	Yes	Not done	Yes	Not done
		E3	Yes	Yes	Yes	Not done
3	Cholesterol A & B ring	3β-HSD & ChoX	Yes	Not done	Not done	Not done
		KstD	Yes	Not done	Not done	Not done
		KshA & KshB	Yes	Not done	Not done	Not done
		HsaA-G	Yes	Yes	Not done	Not done
4.	Cholesterol C & D ring	FadD3	Yes	Not done	Not done	Not done
		ORF18	Yes	Not done	Not done	Not done
5	ß- oxidation	Thiolase	Yes	Yes	Not done	Not done
6	Amino acid degradation	ketoacyl Co-A Transferase	Not done	Not done	Not done	Not done
		keto-butyrate-Co-A Ligases	Not done	Not done	Not done	Not done
7	Krebs cycle	Citrate synthase	Yes	Yes	Not done	Not done
8	Glyxolate shunt pathway	Isocitrate lyases	Yes	Yes	Yes	Not done
		Malate dehydrogenase	Yes	Yes	Not done	Not done
9	Fatty acid synthesis	ACCase	Yes	Yes	Yes	Not done
		FAS complex	Yes	Yes	Yes	Yes

Table 2. Standard drugs against TB with its target and mode of action with co-relation with Ac Co-A

Sl. no.	Drug against TB	Target	Mode of action	Role of Ac Co-A
1	Isoniazid	Cell wall	Activated INH inhibits mycolic acid biosynthesis	Yes* Mycolic acid is complex fatty acid attached with long alcoholic chain whose initial precursor in Ac Co-A
2	Rifampicin	RNA synthesis	Bind to the RNA pol. and inhibits m-RNA synthesis and hence no protein formation	Not done
3	Pyrazinamide	Unknown	Cause non specific damage	Unknown
4	Ethambutol	Cell wall	Prevent arabinogalactan synthesis	Not done
5	Streptomycin	Protein synthesis	Bind to ribosome and inhibits its action	Not done
6	Kanamycin & Amikacin (Aminoglycosides)	Protein synthesis	Bind to ribosome and inhibits its action (binding with 16S of r-RNA subunit)	Not done
	Capreomycin & Viomycin	Protein synthesis	Binds to 50S & 30S ribosomal subunits and inhibits its action	Not done
8	Ethinoamide & Prothionamide	Cell wall	Activated drug causes mycolic acid biosynthesis	Yes [63] Mycolic acid is complex fatty acid attached with long alcoholic chain whose initial precursor in Ac Co-A
9	Ofloxacin & Ciprofloxacin (Fluoroquinolones)	DNA structure replication	Inhibits DNA gyrase and topoisomerase-IV to inhibits supercoiling and replication	Not done
10	Cycloserine	Cell wall	Cell wall biosynthesis (alanine racemase and d-Ala-d-Ala ligase)	Not done
11	p-Aminosalicylic acid	Folate metabolism	Thymidylate synthase inhibition and interference in iron acquisition	Not done

formation of both mycolic acids and lipid virulence factors in mycobacteria. PptT was identified as a new target for anti-tuberculosis drugs; PptT was shown to be essential for the growth of *M. bovis* BCG in vitro and further required for persistence of *M. bovis* BCG in both infected macrophages and immunodeficient mice, which was further confirmed by the on *pptT* mutant of *M. tuberculosis* [59]. All these results demonstrate that PptT is required for the replication and survival of the tubercle bacillus during the acute and chronic phases of infection in mice.

Further, the literature supports of KS as a promising and attractive target for novel anti-TB drugs, designing and discovery because of its distinct and play a vital role in the mycolic acid synthesis, cell wall synthesis, biofilm formation and also pathogenesis [60]. ER can be another potential drug candidate as it thought to be present in single isoform enzyme in the genome of the bacteria, but the study of *fab*I mutant of *P. aeruginosa* and *Bacillus subtilis* was able to survive in vitro condition [61]. Triclosan, a broad spectrum antibacterial product used for the inhibition of ER in fatty acid synthase is quite effective against *M. smegmatis* while *M. tuberculosis* is totally resistant against the action of this drug which can be because of the presence of a specific efflux pump or detoxification process [62].

6. CONCLUSION

Ac Co-A is an important central metabolite responsible for the synthesis of several biomolecules including complex metabolites, hormones, signal molecules and also a major product of common metabolite degradation pathways. Here we analyzed the role of enzymes which are responsible for the degradation and utilization of Ac Co-A for the cellular process as a potential drug target against *M. tb*. This analysis is based on the several information required for the validation of a potential drug target. This validation includes firstly by mutation or deletion of the gene of interest and then the determination of its phenotype; secondly by expression, purification and activity of recombinant enzyme and further its structure determination and final screening of potent inhibitor based on the structure-molecule docking model.

So far fatty acyl Co-A carboxylase and Isocitrate lyases are two well studies enzymes related to drug development against *M. tb* whose knockout studies separately showed that both the enzymes are crucial for the survival of either active or latent stage of *M. tb* within the host macrophages. Henceforth, a number of inhibitors screened showed good efficacy and specificity against ACCs and ICL which are now under clinical trials. On the other hand, enzymes such as Ac Co-A synthetasea crucial enzyme required for the de-novo synthesis of acetyl co-A, has not been analyzed as a potential drug target which could be worthwhile. Although, Allicin, an inhibitor of Ac Co-A synthetase shows promising affects on survival of pathogens indicating the potency of this enzyme against *M. tb* survival [19]. Pyruvate dehydrogenase, another important enzyme is also shown to play an alternative role of scavenging toxic, reactive nitrogen species released by activating host macrophages, , can be further investigated for anti-TB therepay whose role in survival of bacilli inside the host are compromised in case mutant PDA complex subunits Furthermore, cholesterol degradation is an important pathway for the survival of bacilli in hostile condition inside the granuloma requires detailed investigation for its potential role in drug discovery. The mutant studies done either *in vitro* or *in vivo* with the support of enzyme activity clearly established that most of gene involved in either transportation or its degradation is crucial for the survival of bugs. Although further investigations are required in terms of structure elucidation with further screening of inhibitors against theses enzymes which will be surely a fertile area of research which is still unexplored.

The role of fatty acyl Co-A dehydrogenase is well defined in fatty acid degradation and its inhibition showed remarkable improvement during angina and diabetes in human being, but the survival of *fda* mutant *M. tb* inside the human host is yet to be analyzed. While the role of several acyl transferases and ligases are seen to be important during the last stage of amino acids degradation pathway, which indicate the potential role of these enzymes in drug discovery against *M. tb*.

Further, the first enzyme of the Krebs cycle, citrate syntheses showed induced expression under dormancy condition which indicate its role in *M. tb* survival. Although citrate syntheses enzyme validation as potential drug is not done so far, but its structure has been resolved recently, which showed a unique kind of closed and open confirmation during the releasing and binding of Ac Co-A and OAA substrates which can be further analyzed for specific targeting the bug. Another important enzyme, fatty acid synthase provides the fruitful area for drug discovery research as all seven subunits of complex can be the ideal candidate for the novel drug discovery against *M. tb.* We analyzed the co-relation of acetyl Co-A with existing TB drugs which showed that the cell wall and mycolic acid synthesis are directly or indirectly related with acetyl Co-A metabolites, which showed an importance of acetyl Co-A role in drug designing against *M. tb.*

Moreover, in this review we are providing with insight of some relevant enzymes related to an important building block for several metabolic pathways (Ac Co-A) which need to be evaluated in detail for its potential role against the survival of active as well as dormant bacilli inside the human host.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Smith I. *Mycobacterium tuberculosis* Pathogenesis and Molecular Determinants of Virulence. Clin Microbiol Rev. 2003; 16(3):463-96.

2. Al-Anazi KA, Al-Jasser AM, Alsaleh K. Infections Caused by *Mycobacterium tuberculosis* in Recipients of Hematopoietic Stem Cell Transplantation. Front Oncol. 2014;4(4):231-42.
3. TB and HIV/Diagnosis, treatment and deaths. Available: http://www.tbfacts.org/tb-hiv.html
4. Global Tuberculosis Report 2013 - World Health Organization. Available:http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656eng.pdf
5. World TB Day 2015: Gear up to end TB. Available:http://www.who.int/campaigns/tb-day/2015/en/
6. Treatment of tuberculosis: guidelines – 4th ed. WHO/HTM/TB/2009.420. Available:http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf?ua=1
7. 2013 Manual of Procedures for the National TB Control Program. Available:http://thepafp.org/wp-content/downloads/cpg/ntp2013.pdf
8. Singh JA, Bhan A, Upshur R. Diagnosis of drug-resistant TB and provision of second-line TB treatment in India: Some ethical considerations. Ind J Med Ethics. 2010;10(4):110-4.
9. Mitnick CD, Shin S, Seung KJ, Rich ML. Comprehensive Treatment of Extensively Drug-Resistant Tuberculosis. N Engl J Med. 2008;359(5):563-84.
10. Rowland K. Totally drug-resistant TB emerges in India Discovery of a deadly form of TB highlights crisis of 'Mismanagement' Nat NEWS; 2012.
11. Long JW. Essential Guide to prescription Drugs 1992 New York: Harper Collins Publishers. 1991;4:925-9.
12. Amy CA. The Process of Structure-Based Drug Design. Chem Biol. 2003;10(1): 787-97.
13. Marrero J, Rhee KY, Schnappinger D, Pethe K, Ehrt S. Gluconeogenic carbon flow of tricarboxylic acid cycle intermediates is critical for *Mycobacterium tuberculosis* to establish and maintain infection. PNAS-USA. 2010;107(2):9819-24.
14. Griffin JE, Pandey AK, Gilmore SA, Mizrahi V, McKinney JD, Bertozzi CR, Sassetti CM. Cholesterol catabolism by *Mycobacterium tuberculosis* requires transcriptional and metabolic adaptations. Chem Biol. 2012; 19(3):218-27.
15. Khan A, Sarkar D. Nitrate reduction pathways in mycobacteria and their implications during latency. Microbiology. 2011;158(2):301-7.
16. Akhtar S, Khan A, Sohaskey CD, Jagannath C, Sarkar D. Nitrite Reductase NirBD is induced and plays an important role during *in vitro* dormancy of *Mycobacterium tuberculosis.* J Bacteriol. 2013;195(20): 4592-9.
17. Berg JM. The Citric Acid Cycle Oxidizes Two-Carbon Units. Biochemistry, 5th Edition; 2002.
18. Ingram-Smith C, Smith KS. AMP-forming acetyl-CoA synthetases in show unexpected diversity in substrate utilization. Archaea. 2007;2(2):95-107.
19. Focke M, Feld A, Lichtenthaler K. Allicin, a naturally occurring antibiotic from garlic, specifically inhibits acetyl-CoA synthetase. FEBS Letters. 1990;261(1):106-8.
20. Schreiner ME, Fiur D, Holátko J, Pátek M. E1 Enzyme of the Pyruvate Dehydrogenase Complex in *Corynebacterium glutamicum*: Molecular analysis of the gene and phylogenetic aspects. J Bacteriol. 2005; 187(4):6005-8.
21. Venugopal A, Bryk R, Shi S, Rhee K. Virulence of *Mycobacterium tuberculosis* depends on lipoamide dehydrogenase, a member of three multi-enzyme complexes. Cell Host Microbe. 2011;9(4):21-31.
22. Shi S, Ehrt S. Dihydrolipoamide acyltransferase is critical for *Mycobacterium tuberculosis* pathogenesis. Infect Immun. 2006;74(1):56-63.
23. Rajashankar KR, Bryk R, Kniewel R, Buglino JA, Nathan CF, Lima CD. Crystal structure and functional analysis of lipoamide dehydrogenase from *Mycobacterium tuberculosis.* J Biol Chem. 2005;280(4):33977-83.
24. Bryk R, Arango N, Maksymiuk C, Balakrishnan A, et al. Lipoamide channel-binding sulfonamides selectively inhibit mycobacterial lipoamide dehydrogenase. Biochemistry. 2013; 52(51):9375-84.
25. Jeyaseelan K, Guest JR. Isolation and Properties of Pyruvate Dehydrogenase Complex Mutants of *Pseudomonas aeruginosa* PAO. J Gen Microbiol. 1980; 120(2):385-92.
26. Chandrasekhar K, Arjunan P, Sax M, Nemeria N, Jordan F, Furey W. Active-site changes in the pyruvate dehydrogenase multienzyme complex E1 apoenzyme component from *Escherichia coli* observed at 2.32 A resolution. Acta

Crystallogr D Biol Crystallogr. 2006;62(11): 1382-6.
27. Russell DG, Cardona PJ, Kim MJ, Allain S, Altare F. Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol. 2009;10(9):943-8.
28. Pandey AK, Sassetti CM. Mycobacterial persistence requires the utilization of host cholesterol. PNAS-USA. 2008;105(11): 4376-80.
29. Klink M, Brzezinska M, Szulc I, Brzostek A, et al. Cholesterol oxidase is indispensable in the pathogenesis of *Mycobacterium tuberculosis*. PLoS-One. 2013;8:e73333.
30. Beste DJ, Noh K, Niedenfuhr S, Mendum TA, et al. 13C-flux spectral analysis of host-pathogen metabolism reveals a mixed diet for intracellular *Mycobacterium tuberculosis*. Chem Biol. 2013;20(8):1012-21.
31. Griffin JE, Gawronski JD, Dejesus MA, Ioerger TR, Akerley BJ, Sassetti CM. High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. PLoS Pathog. 2011;7(9):e1002251.
32. Hu Y, Van Der Geize R, Besra GS, Gurcha SS, Liu A, Rohde M, Singh M, Coates A. 3-Ketosteroid 9alpha-hydroxylase is an essential factor in the pathogenesis of *Mycobacterium tuberculosis*. Mol Microbiol. 2010;75(1):107-121.
33. Dresen C, Lin LY, D'angelo I, Tocheva EI, Strynadka N, Eltis LD. A flavin-dependent monooxygenase from *Mycobacterium tuberculosis* involved in cholesterol catabolism. J Biol Chem. 2010;285(9); 22264-75.
34. Rengarajan J, Bloom BR, Rubin EJ. Genome-wide requirements for *Mycobacterium tuberculosis* adaptation and survival in macrophages. PNAS-USA. 2005; 102(23):8327-32.
35. Carere J, Mckenna SE, Kimber MS, Seah SY. Characterization of an aldolase-dehydrogenase complex from the cholesterol degradation pathway of *Mycobacterium tuberculosis*. Biochemistry 2013;52(20):3502-11.
36. Casabon I, Crowe AM, Liu J, Eltis LD. FadD3 is an acyl-CoA synthetase that initiates catabolism of cholesterol rings C and D in actinobacteria. Mol Microbiol. 2013a;87(2):269-283.
37. Horinouchi M, Hayashi T, Koshino H, Kudo T. ORF18-disrupted mutant of *Comamonas testosteroni* TA441 accumulates significant amounts of 9, 17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-oic acid and its derivatives after incubation with steroids. J Steroid Biochem Mol Biol. 2006;101(1): 78-84.
38. Lodish H. Section 16.1Oxidation of Glucose and Fatty Acids to CO_2. Mol Cell Biol. 4th Edition; 2000.
39. Anbazhagan P, Harijan RK, Kiema TR, Janardan N, et al. Phylogenetic relationships and classification of thiolases and thiolase-like proteins of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis.* Tuberculosis 2014;94(4): 405-412.
40. Janardan N, Harijan RK, Wierenga RK, Murthy MRN. Crystal Structure of a Monomeric Thiolase-Like Protein Type 1 (TLP1) from *Mycobacterium smegmatis*. PLoS-ONE. 2012;7(7):e41894.
41. Ayub ND, Julia Pettinari M, Méndez BS, López NI. Impaired polyhydroxybutyrate biosynthesis from glucose in *Pseudomonas sp.* 14-3 is due to a defective ß-ketethiolase gene. Microbiol Lett. 2006;264(1):125-31.
42. Lee HJ, Choi MH, Kim TU, Yoon SC. Accumulation of Polyhydroxyalkanoic Acid Containing Large Amounts of Unsaturated Monomers in *Pseudomonas fluorescens* BM07 Utilizing Saccharides and Its Inhibition by 2-Bromooctanoic Acid. Appl Environ Microbiol. 2001;67(4):114963-74.
43. Nelsion DL, Cox MM. Amino Acid Oxidation and the Production of Urea-Chapter 18," Leninger: Principle of Biochemistry 4th Edition; 2004.
44. Li BH, Ma XF, Wu XD, Tian WX. Inhibitory Activity of Chlorogenic Acid on Enzymes Involved in the Fatty Acid Synthesis in Animals and Bacteria. IUBMB Life. 2006; 58(1):39-46.
45. Singh V, Mani I, Chaudhary DK, Somvanshi P. The β-ketoacyl-ACP synthase from *Mycobacterium tuberculosis* as potential drug targets. Curr Med Chem. 2011;18(12):1318-24.
46. Duckworth BP, Nelson KM, Aldrich CC. Adenylating enzymes in *Mycobacterium tuberculosis* as drug targets. Curr Top Med Chem. 2012;12(7):766-796.
47. Tian J, Bryk R, Itoh M, Suematsu M, Nathan C. Variant tricarboxylic acid cycle in *Mycobacterium tuberculosis*: Identification of α-ketoglutarate decarboxylase. PNAS-USA 2005;102(30): 10670–5.
48. Vandedrinck S, Deschamps G, Sablon E, Vandamme EJ. Metabolic engineering

of *Escherichia coli*: Construction and characterization of a *gltA* (citrate synthase) knockout mutant. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet. 2001;66(3a):333-6.

49. Ferraris DM, Spallek R, Oehlmann W, Singh M, Rizzi M. Structures of citrate synthase and malate dehydrogenase of *Mycobacterium tuberculosis*. Proteins. 2015;83(2):389-94.
50. Asif M. Role of malate synthase and other enzyme inhibitors in *Mycobacterium Tuberculosis*: A mini review. Biotech, Mol Biol Nanomed. 2013;1(1):35-8.
51. Sharma V, Sharma S, Hoener ZU, Bentrup K, McKinney JD, Russell DG, Jacobs Jr WR, Sacchettini JC. Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis*. Nat Struct Biol. 2000;7(11):663-8.
52. Krátký M, Vinšová J. Advances in mycobacterial isocitrate lyase targeting and inhibitors. Curr Med Chem. 2012; 19(36):6126-37.
53. Karakousis PC, Bishai WR, Dorman SE. *Mycobacterium tuberculosis* cell envelope lipids and the host immune response. Cell Microbiol. 2004;6(2):105-16.
54. Zhang X, Guo J. Advances in the treatment of pulmonary tuberculosis. Thoracic Dis. 2012;4(3):617-23.
55. Tong L. Acetyl-coenzyme a carboxylase: Crucial metabolic enzyme and attractive target for drug discovery. Cell Mol Life Science. 2005;62(11):1784-95.
56. Freiberg C, Brunner NA, Schiffer G, Lampe T, Pohlmann J, et al. Identification and characterization of the first class of potent bacterial acetyl-CoA carboxylase inhibitors with antibacterial activity. J Biol Chem. 2004;279(3):26066-73.
57. Lin TW, Melgar MM, Kurth D, Swamidass SJ, Purdon J, et al. Structure-based inhibitor design of AccD5, an essential acyl-CoA carboxylase carboxyltransferase domain of *Mycobacterium tuberculosis.* PNAS-USA. 2006;103(9):3072-7.
58. Singh V, Mani I, Chaudhary DK, Somvanshi P. The β-ketoacyl-ACP synthase from *Mycobacterium tuberculosis* as potential drug targets. Curr Med Chem. 2011;18(9):1318-24.
59. Leblanc C, Prudhomme T, Tabouret G, Ray A, Burbaud S, et al. 4'-Phosphopantetheinyl Transferase PptT, a new drug target required for *Mycobacterium tuberculosis* growth and persistence *In vivo*. PLoS Pathog. 2012;8(12):e1003097.
60. Brown AK, Taylor RC, Bhatt A, Fütterer K, Besra GS. Platensimycin activity against mycobacterial β-Ketoacyl-ACP synthases. PLoS-ONE. 2009;4(7):e6306.
61. Heath RJ, Su N, Murphy CK, Rock CO. The enoyl-[acyl-carrier-protein] reductases, FabI and FabL, from *Bacillus subtilis.* J Biol Chem. 2000;275(2):40128-33.
62. Bhargava HN, Leonard PA. Triclosan: Applications and safety. Am J Infect Control. 1996;24(9):209-18.
63. Schroeder EK, De Souza N, Santos DS, Blanchard JS, Basso LA. Drugs that inhibit mycolic acid biosynthesis in *Mycobacterium tuberculosis*. Curr Pharm Biotechnol. 2002; 3(3):197-25.

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