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Characterization of 3β-hydroxysteroid dehydrogenase (*Rv1106c*) and the sterol metabolic pathway in *Mycobacterium tuberculosis* (*M. tb*)

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Abstract of the Dissertation

Characterization of 3β-hydroxysteroid dehydrogenase (*Rv1106c*) and the sterol metabolic pathway in *Mycobacterium tuberculosis* (*M. tb*)

by
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Mycobacterium tuberculosis (M. tb), the causative agent of tuberculosis, has infected approximately one third of the world's population. *M. tb* is an intracellular pathogen that shifts to a lipid based metabolism in the host environment. Cholesterol metabolism is proposed to be important for mycobacterial persistence in the host. Therefore, the biochemical functions of several genes associated with the pathway were investigated to identify new targets of this pathogen.

3β-Hydroxysteroid dehydrogenase (*hsd*, *Rv1106c*) is required for the growth of *M. tb* on cholesterol as a sole carbon source and the disruption of *hsd* in *M. tb* abrogates mycobacterial cholesterol oxidation activity, consistent with the *Rv1106c* gene product being the only enzyme responsible for 3β-hydroxysterol oxidation in *M. tb*. The *hsd* gene was cloned and heterologously expressed. The purified enzyme was kinetically characterized and found to use NAD^+ as a cofactor, and oxidize cholesterol, pregnenolone, and dehydroepiandrosterone to their respective 3-keto-4-ene products. The enzyme shows no substrate preference for dehydroepiandrosterone versus

pregnenolone. Trilostane is a competitive inhibitor of dehydroepiandrosterone and NADH was found to be a product inhibitor of the reaction. Analysis of the two substrate kinetics and the inhibitory pattern of trilostane and NADH, revealed that the enzyme forms a ternary complex with NAD^+ binding before the sterol.

Apolar lipid profiles of wild-type *M. tb* cells with or without cholesterol as a carbon source were obtained, using electrospray ionization on an LTQ Orbitrap mass spectrometer. The mass of phthiocerol dimycocerosate shifted to a higher molecular weight with cholesterol as a sole carbon source. This result supports the idea that cholesterol can be metabolized to propionate in addition to acetate.

Based on the genomic profiling and studies of sterol metabolic pathways in mycobacteria and other bacteria, a cholesterol metabolic pathway in *M. tb* has been proposed. The ring degradation starts from the oxidation of 3β-hydroxysterol by *hsd*, and follows further A/B ring degradation or side chain degradation with several rounds of β-oxidation. We found that the wild-type strain, H37Rv, metabolizes cholesterol to androst-4-ene-3,17-dione and androsta-1,4-diene-3,17-dione and exports these metabolites into the medium. The *fadA5* (*Rv3546*) mutant strain, which is required for utilization of cholesterol as a sole carbon source and also for full virulence, is defective for this activity. These results are consistent with a role for FadA5 in the thiolysis activity required for β-oxidation. The inability of the *fadA5* mutant to produce AD and ADD may be related to its attenuated virulence *in vivo*.

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List of Schemes

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2xYT	2×yeast-tryptone broth
3-HSA	3-hydroxy-9,10-seconandrost-1,3,5(10)triene-9,17-dione
3,4-DHSA	3-hydroxy-9,10-seconandrost-1,3,5(10)triene-9,17-dione
4,9-DHSA	4,5-9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10), 2-diene-4-ioc acid
9-OH-ADD	9α-hydroxy-androsta-1,4-diene-3,17-dione
AD	androst-4-ene-3,17-dione
ADD	androsta-1,4-diene-3,17-dione
APCI (API)	atmospheric pressure chemical ionization
BSA	Bovine serum albumin
$CHCl_3$	chloroform
CID	collision induced dissociation
COSY	correlation spectroscopy
DEAE	diethylaminoethyl
DHEA	dehydroepiandrosterone
DOHNAA	9,17-dioxo-1,2,3,4,10,19-hexanoandrostan-5-ioc acid
ESCI	multi-mode ionization source with API and ESI
ESI	electrospray ionization
FAD	flavin adenine dinucleotide
FadA5	acetylCoA acetyltransferase
FadE29	acylCoA dehydrogenase
HMBC	heteronuclear multiple bond coherence
HsaA	3-HAS hydroxylase
Hsd	3β-hydroxysteroid dehydrogenase
HSQC	heteronuclear single quantum coherence

igr	intracellular growth
IMAC	immobilized-metal ion affinity chromatography
IPTG	isopropyl β-D-thiogalactoside
K_{app}	Apparent equilibrium constant
k_{cat}	Michaelis-Menten catalytic rate constant
K_d	Dissociation constant
kD	kiloDalton
K_m	Michaelis-Menten constant
LB	Luria broth
MALDI	matrix-assisted laser desorption ionization
MIC	Minimum inhibitory concentration
M. tb	*Mycobacterium tuberculosis*
MDR-TB	multi-drug resistant *Mycobacterium tuberculosis*
NAD^+	β-nicotinamide adenine dinucleotide
NADH	β-nicotinamide adenine dinucleotide, reduced form
NADP	β-nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
ORF	Open reading frame
PCR	Polymerase chain reaction
PDB	Protein data bank
PDIM	Phthiocerol dimycocerosate
R. equi	*Rhodococcus equi*
r3βHSD	recombinant 3β-hydroxysteroid dehydrogenase without an N-terminal six histidine tag
$rH_6$3βHSD	recombinant 3β-hydroxysteroid dehydrogenase with an N-terminal six histidine tag

SIR	selected ion recording
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
TAPS	N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid
UV	ultra violet
V_i	Initial velocity
WT	wild-type
XDR-TB	extremely-drug resistant *Mycobacterium tuberculosis*

Chapter 1

Introduction

I. *Mycobacterium tuberculosis* and pathogenesis

Overview of TB and the emergence of MDR/XDR TB

Mycobaterium tuberculosis (*M. tb*) is a highly contagious microorganism which causes human tuberculosis (TB) and is transmitted via air. TB is a major global health problem. It is estimated that *M. tb* infects one third of the world's population (*1*), and 10% of the infected people develop an active form of TB. Tuberculosis kills 5000 people every day and nearly 2 million each year (*2*). Furthermore, suppression of the immune system, for example, through HIV infection, promotes the likelihood of people becoming infected with TB and the progression of latent TB to active disease. The World Health Organization estimated that of the 9.27 million new cases of TB that occurred in 2007, nearly 15% are coinfected with HIV (*3*). Almost one-third of HIV/AIDS infected people are co-infected with TB and half of all AIDS patients die of TB infection.

TB treatment requires long courses of multiple antibiotics that lead to patients abandoning the regimen before completion of treatment. Incomplete treatment in turn leads to drug resistance and an increased TB burden in the population. Multi-drug resistant TB (MDR-TB) is a specific form due to a bacillus resistant to at least isoniazid and rifampicin, the two front line anti-TB drugs (*4*). Stepwise accumulation of random mutations in the chromosome selected under the environmental pressure of chemotherapy is thought to be the reason for multi-drug resistance (*5*). Extensively drug-resistant tuberculosis (XDR-TB) is defined as TB which is resistant to the front line drugs, plus resistant to any quinolines and at least one of three second-line drugs: amikacin, kanamycin, or capreomycin (*6*-*7*). Treatment options are seriously limited since XDR-TB is resistant to both first- and second-line drugs. New approaches are required to defeat both sensitive and drug resistant strains of *M. tb*.

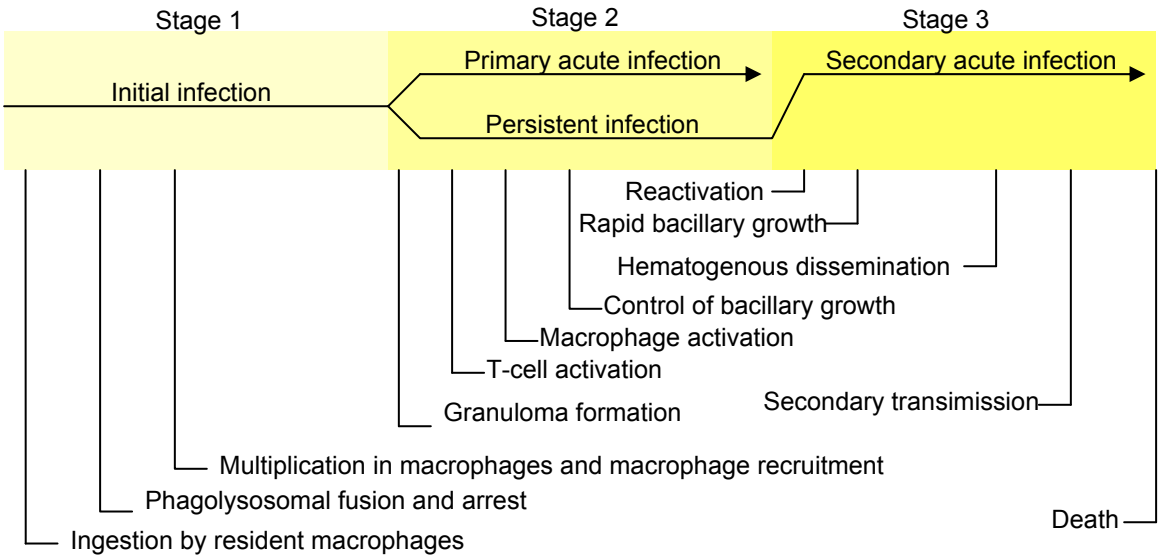
Overview of M. tb

M. tb is an aerobic, rod-shaped, and Gram-positive bacterium (*8*). The cell wall of *M. tb* is composed of suger and lipids including mycolic acids, acyl glycolipids, sulfolipids and other complex free lipids (*9*). The unique mycolic acid-based outer lipid bilayer accounts for its low permeability and resistance to antibiotics as well as the host macrophage's bactericidal reactions (*10*). The biosynthesis of the cell wall is a potential target for anti-TB drug and vaccine development *(11)*.

One milestone in TB research is the publishing of the entire genome sequence of a virulent strain *M. tb* H37Rv in 1998 (*12*), with approximately 4000 open reading frames (ORF) identified. In addition, the proteome of *M. tb* H37Rv has been studied by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) and up to 300 proteins have been identified successfully. Selected proteins were identified by either MALDI-MS or immunodetection (*13-14*). Many proteins were identified to be related to small molecule metabolism. These small molecules include a diverse array of structures, such as fatty acid, isoprenoid, highly complex molecules and the phenolphthiocerol alcohols (*12*). Presently, around 52% of the proteins have been assigned function and 376 putative proteins share no homology with known proteins, i.e., they are unique to *M. tb* (*15*). 35% of ORFs in *M. tb* are predicted to be essential by a postgenomic *in silico* approach (*16*). Additional strategies in comparative, functional and structural genomics, transcriptomics, proteomics, and systems biology have been employed for new drug target identification (*17-18*).

M. tb infection process

The pathogenicity of *M. tb* mainly relies on its survival ability in macrophages, and the overall infection process includes three separate stages (*19*) (Scheme 1-1).



Scheme 1-1. Stages of *M. tb* persistent infection (*19*).

In the first stage, the infectious *M. tb* is inhaled as droplets from the atmosphere. Within the lungs, the bacilli infect and reside in phagocytic cells such as alveolar macrophages and dendritic cells. After the phagocytosis, the macrophages are generally thought to act as an effective barrier to the pathogens by numerous mechanisms, including phagosome-lysosome fusion, and generation of reactive oxygen and nitrogen intermediates. However, *M. tb* has evolved diverse strategies to mainly perturb the phagosomal maturation (*20*) in order to survive and spread within the cells, such as reducing the acidification of *M. tb* containing phagosomes by ammonia production (*21*) and H^+-ATPase exclusion (*22*), modifying the normal phagosomal trafficking pathway, or altering the recruitment or association of various phagosomal membrane proteins (*23*). *M. tb* also uses a range of protein and lipid effectors, such as phosphatidylinositol mannoside (PIM) and lipoarabinomannan (LAM), to alter phosphatidylinositol-3-phosphate (PI(3)P) signaling (*24*). *M. tb* further impairs cytosolic Ca^{2+} flux and increases Ca^{2+} efflux from the ER by inhibiting sphingosine kinase, which converts sphingosine to sphingosine-1-phosphate (*23, 25*). A genetic screen for *M. tb*

mutants indicated genes encoding components in ESX-1 secreted system, lipid synthesis and secretion, and molybdopterin biosynthesis are involved in modulating trafficking within the host (*26*).

Collectively, macrophages provide an environment for both cellular defense and bacterial replication. Before the macrophages are immunologically activated, the phagocytosed bacilli replicate inside macrophages (*27*). The bacilli released can then infect other infiltrating peripheral macrophages.

The second stage of *M. tb* infection is characterized by a cell-mediated host's immune response. An acute infection will occur with an immunocompromised host, following the uncontrolled bacillary replication and dissemination of *M. tb*. Alternatively, the immune system will resolve the initial infection, or in most cases result in a persistent infection, if the infected host is immunocompetent (Scheme 1-1).

The immune response includes T-cell activation, both conventional $CD4^+$ and $CD8^+$ T cells and unconventional T cells, such as γδ T cells, and double-negative or CD4/CD8 single positive T cells. During the differentiation of $CD4^+$ T cells into T-helper-1 (Th1) cells, dendritic cell (the most efficient antigen presenting cells) (*28*) can produce IL-12, IL18 and co-stimulatory molecules (*29-30*), which in turn stimulate T-cell activation. Th1 cells then secrete their own lymphokines, such as interferon-γ (IFN-γ). Macrophages secrete proinflammatory cytokines, such as IL-10 and tumor-necrosis factor-α (TNF-α) (*31-32*). IFN-γ and TNF-α are central factors in the macrophage activation. Macrophage activation leads to lysosome maturation and produces antimicrobial molecules such as reactive nitrogen intermediates (RNI) and reactive oxygen intermediates (ROI) (*33*). *M. tb*, on the other side, employs mechanisms to evade these factors and maximize its survival. The inhibition of IL-12 production is one key mechanism for *M. tb* survival (*34*). Glycolipid trehalose 6,6′-dimycolate (TDM) has been identified as an effector molecule for the repression of IL-12p40 production.

MmaA4 gene, encoding a methyl transferase, is required for introducing the distal oxygen-containing modifications of mycolic acid. Recent studies identified mmaA4 as a key locus involved in the repression of IL-12 production (*35*). Phenolic glycolipids (PGL) were also determined to directly modulate the host innate immune response (*36*).

The localized proinflammatory response leads to the release of mononuclear cells and therefore the formation of granulomas (*37*), the site of infection, persistence, pathology and protection. More mature phase granulomas show neovascularization and develop a fibrotic capsule. Granulomas are composed of infected macrophages surrounded by foamy macrophages and other organized immune cells that surround foci of infected tissues. Oxygenated mycolic acids induce the differentiation of mice macrophages into foamy macrophages (*38*). Foamy macrophages were observed to be surrounded with lipid droplets in lung, in both human infection and mouse model of infection. The lipid bodies, confirmed by electron microscopy, were observed to interact with *M. tb* bacilli (*39*). The accumulation of lipids within bacilli, via interaction with foamy macrophage lipid bodies could be crucial to *M. tb* persistence, and the formation of foamy macrophages is important to the further necrosis.

The third stage of infection is mainly indicated by the reactivation of bacillus from latency and therefore the initiation of a secondary acute infection, probably triggered by the change in immune system, in particular, HIV or poor nutrition or environmental stress. The mature granulomas lose their vascular appearance and further break down. The bacteria are then released into the airways, leading to transmission. The mechanisms that allow latent TB are not fully characterized yet.

During the whole process of infection, the lipid metabolism in both pathogen and host shows critical importance. Although lipid synthesis and the relationship to the infection in the pathogen have been partially studied, host lipid metabolism and how the host lipid affects infection are not clear. Therefore the understanding of these

mechanisms may ultimately help better development of new therapies.

II. Cholesterol metabolism in *M. tb* and other organisms

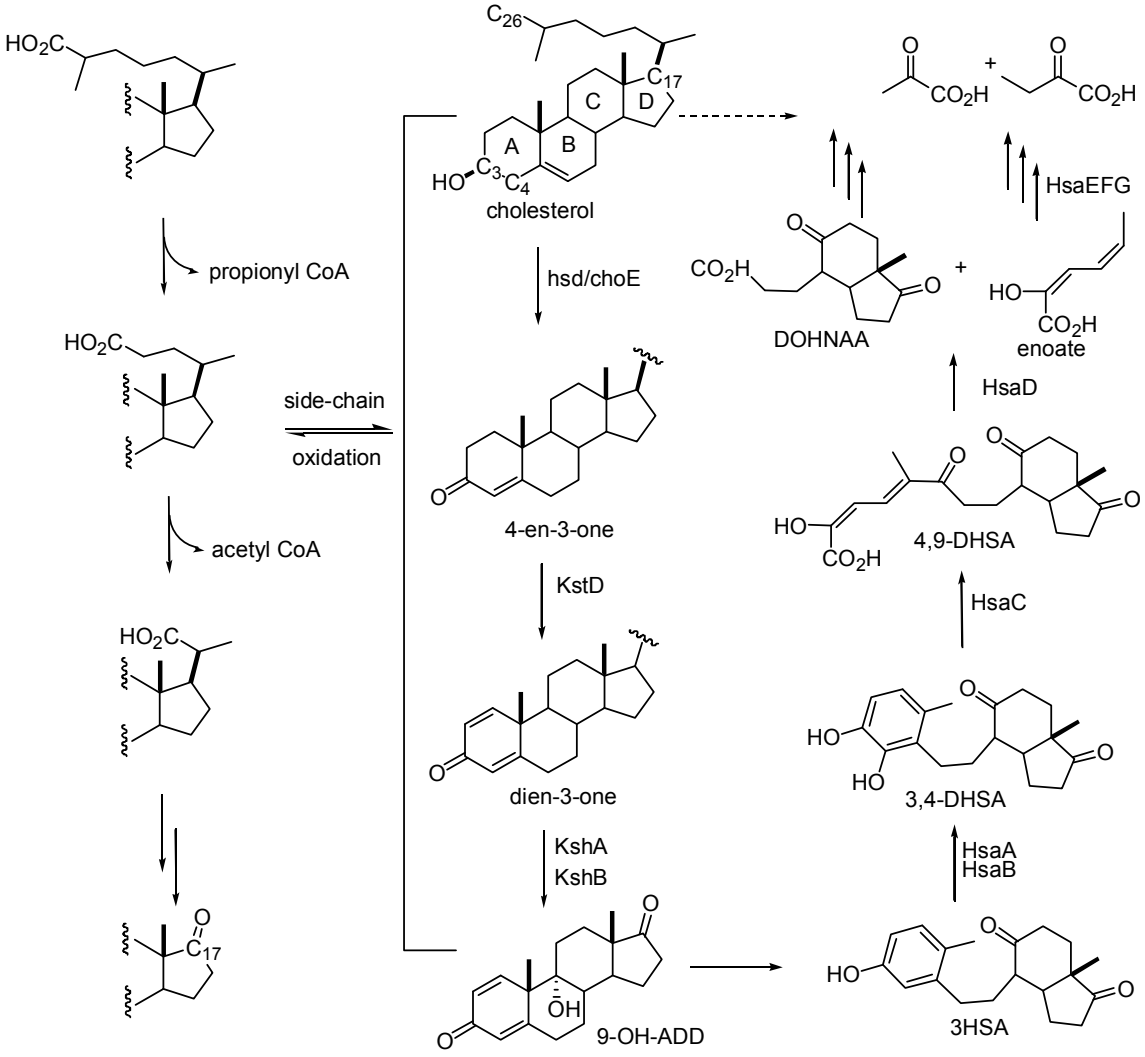
Cholesterol and bacteria

Cholesterol is found in all animal cells and is primarily a component of membranes of these cells. The content of cholesterol often determines the function of a membrane and cholesterol helps to maintain integrity, fluidity and important proteins in the membrane. On the contrary, many actinomycetes and mycobacteria lack cholesterol in their membranes, however, they possess the ability to metabolize cholesterol and even use cholesterol as a single carbon source. In addition, cholesterol has long been identified as a source of steroid hormones, important immune system factors, bile acids and Vitamin D.

Cholesterol biosynthesis in eukaryotic cells has been fully mapped out, with hydroxymethylglutaryl-coenzyme A (HMG-CoA) as a precursor. Moreover, microbial cholesterol metabolism studies attracted considerable attention on finding metabolites suitable for low-cost pharmaceutical starting materials of bioactive steroids and enzymatic activities useful in synthetic transformation of steroid (*40-41*).

Metabolites of cholesterol have been identified in several actinomycete strains. The catabolism includes ring degradation and side-chain degradation, simultaneously or in a certain order. The steroid is sequentially oxidized and dehydrogenated to propionic and acetic acid. *Rhodococcus equi*, a bacterium related to *M. tb*, is an opportunistic pathogen that infects immune compromised individuals. And *Rhodococcus* species are well known for their catabolic potential to degrade natural phytosterols (*42*). A group of genes in *Rhodococcus sp. RHA1* was reported to be upregulated by cholesterol. There are 58 genes of the *ro00482-ro04705* cluster, with the proposed function of cholesterol

catabolism, conserved within an 82 gene cluster in *M. tb H37Rv* (*Rv3492c-Rv3574*), *M. bovis bacillus Calmette-Guérin* (*Bcg3556c-Bcg3639*) and an 80-gene cluster in *M. avium* (*Map0571-Map0491*) (*43-44*). The metabolism of cholesterol in mycobacteria may potentially relate to bacterial nutrients or virulence. The proposed pathway is shown in Scheme 1-2.



Scheme 1-2. Proposed cholesterol degradation pathway based on studies in *Rhodococcus (45), C. testosteroni (46-47) and M. smegmatis (48)*. Abbreviations: 9-OH-ADD (9α-hydroxy-androsta-1,4-diene-3,17-dione); 3-HSA (3-hydroxy-9,10-seconandrost-1,3,5(10)triene-9,17-dione); 3,4-DHSA (3-hydroxy-9,10-seconandrost-1,3,5(10)triene-9,17-dione); 4,9-DHSA (4,5-9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10),2-diene-4-ioc acid); DOHNAA (9,17-dioxo-1,2,3,4,10,19-hexanoandrostan-5-ioc acid) .

The enzymes catalyzing the conversion of 3β-hydroxysteroid to 3-ketosteroid belong to two enzyme families: oxidase and dehydrogenase. Both catalyze the same overall chemical reactions: oxidation and isomerization. *Rhodococcus (49)* and *Streptomyces (50)* use cholesterol oxidases, which require an FAD cofactor regenerated by the reduction of O_2 to H_2O_2. Cholesterol oxidase associates with lipid bilayers and the conversion of cholesterol to cholest-4-en-3-one increases the disorder of a membrane and leads to the intracellular contents leakage or even the membrane lysis (*51-53*). *Nocardia (54)*, *proteobacteria (55)* and *C. testosteroni (56)* utilize dehydrogeanses, using NAD(P) as an oxidizing cofactor.

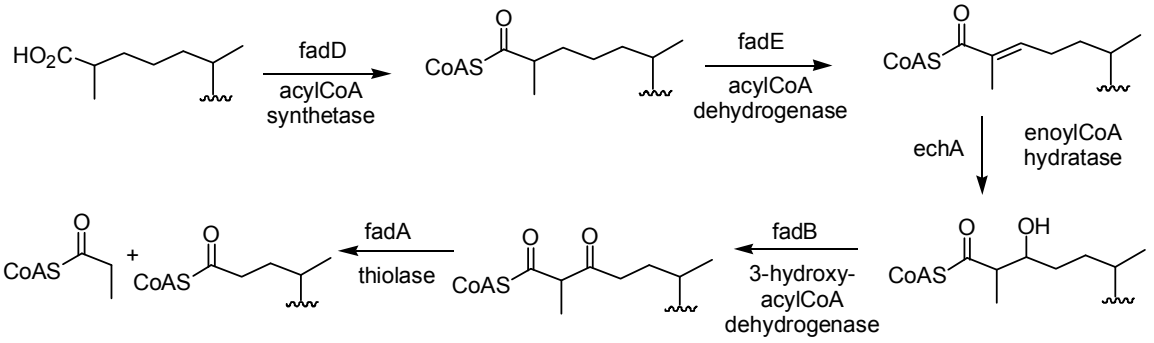
The further A/B ring degradation has been characterized to different extents. In *Rhodococcus* and *M. smegmatis*, the next step of ring degradation is dehydrogenation to dien-3-one by ketosteroid Δ^1-dehydrogenase (*kstD*, also known as *ksdD*) (*57-59*) . Two isomers were identified for each organism and their respective genes have been cloned and expressed and knockout experiments confirmed the function of *kstD*. And the significant accumulation of androstenedione (a substrate of *kstD*) by *M. smegmatis* Δ*ksdD-1* induced by cholesterol suggests higher KsdD activity of one isomer over another (*60*). The following step is 9-hydroxylation catalyzed by KshA/B (a two-component class IA oxygenase) to yield 9α-hydroxyandrost-1,4-diene-3,17-dione, and a spontaneous ring opening to form 3-hydroxy-9,10-secoandrost-1,3,5(10)-triene-9,17-dione (3-HSA). KshA/B are characterized in *Rhodococcus* (*45*) and *M. smegmatis(48)*. Disruption of the *kshA/B* gene prevents *Rhodococcus* growth on androstenedione or androstadienedione as a sole carbon source (*45*). At least three sets of *kshA* homologs were found in *Rhodococcus erythropolis strain SQ1*, indicating different pathways may apply to distinct sterol degradation (*61*).

The next step is the hydroxylation of the aromatic A ring by the annotated meta-oxygenase HsaA/HsaB. Phenol 3HSA is converted to 3,4-dihydroxy-9,10-seconandrost-

1,2,3-triene-9,17-dione (3,4-DHSA). *tesA1* and *tesA2* in *C. testosteroni* are homologs of *hsaA/B* in *Rhodococcus.* The accumulation of two characteristic intermediate compounds, 3-HSA and its hydroxylated derivative by *tesA1/2*-disrupted mutant confirmed their function (*62*). Then HsaC, the homolog of TesB in *C. testosteroni*, catalyzes the formation of 4,5-9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10),2-diene-4-oic acid (4,9-DHSA). 4,9-DHSA is split by a *meta*-cleavage dioxygenase to 9,17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-oic acid (DOHNAA) and enoate. TesD in *C. testosteroni (46)* is characterized and catalyzes this reaction. *hsaD* in *R. RHA1* is the homolog of *tesD*. Beside *tesD*, there is a steroid degradation gene cluster consisting of *ORF18, 17, H, A2, A1*, and *tesDEFG* in *C. testosteroni.* Gene knockout and metabolite studies have been performed with the *C. testosteroni* strain, confirming that TesE/F/G catalyze reactions downstream of TesD (*47, 63*). Their corresponding genes code for a 2-hydroxypentadienoate hydratase, a 4-hydroxy-2-oxovalerate aldolase and a propionaldehyde dehydrogenase, respectively. In *R. RHA1*, HsaE/F/G are annotated to catalyze the reaction of enoate to pyruvate and propionate based on their homology to the *C. testosteroni* gene cluster. The enzymes and genes for conversion of DOHNAA have not been identified. A *fadE28* gene in *R. RHA1* and *C. testosteroni,* is proposed to catalyze the dehydrogenation of hexahydroindanone (*44*).

The identities of the genes coding for side-chain degrading proteins are unknown. The first step is oxidation of the C26(27) methyl to a carboxylic acid. It is widely assumed that a cytochrome P450 enzyme is responsible for this oxidation, although no genes have been identified in any actinomycete to date. One candidate in *Streptomyces* is ChoP, a cyt P450 in an operon with the cholesterol oxidase gene ChoE, however its function is not determined yet (*64*). In the aerobic pathway, the side chain is then degraded via two rounds of β-oxidation and one retro-aldol reaction. After formation of the thioester with a CoA ligase (FadD), one cycle of β-oxidation requires four enzymes:

an acyl CoA dehydrogenase (FadE), an enoyl CoA hydratase (EchA), a 3 hydroxy acyl CoA dehydrogenase (FadB or short chain alcohol dehydrogenase), and a thiolase (FadA) (Scheme 1-3). A third round of β-oxidation cannot occur due to the secondary carbon at the β-position. A nonenzymatic retroaldol reaction or an α-oxidation may proceed as the next step. It is difficult to assign β-oxidation genes to this pathway, since numerous sets of β-oxidation genes were annotated in actinomycetes (*44*), similar to *M. tb*.



Scheme 1-3. Proposed side-chain degradation pathway through β-oxidation.

In *S. denitrificans*, cholesterol can be converted finally to CO_2 in the absence of oxygen (*65*). Initial steps in the anaerobic cholesterol metabolism system are annotated differently. Cholesterol undergoes the oxidation of A ring, with the first two genes (AcmA and AcmB) confirmed as dehydrogenase and ketosteroid Δ^1-dehydrogenase, respectively. It is followed by C25 hydroxylation with water not oxygen, with a molybdenum enzyme proposed (*66-67*). The subsequent steps are not identified.

Cholesterol and M. tb

The genome sequencing of *M. tb* indicated the abundance of genes involved in fatty acid and lipid metabolism (*12*). During intracellular growth and chronic infection, *M. tb* uses host fatty acid rather than carbohydrate as dominant carbon substrates (*68-69*). Fatty acids could be obtained by hydrolysis of lipids in the phagosomal membrane (*70*). Lipid metabolism plays a crucial role during *M. tb* infection (*35-36, 71*). It is

hypothesized that host-derived lipids provide nutrient sources and *M. tb* derived lipids mediate host immune response (*72*).

Cholesterol is abundant in the intracellular environment, and is important in the mycobacterial uptake by macrophages (*73*) and persistence during chronic infection (*74*). The initial attempts to grow *M. tb*, bacillus Calmette-Guerin (BCG) or other pathogenic mycobacteria on cholesterol as a sole carbon source were not successful (*75*). However, further studies revealed the growth curve reaches stationary phase at much lower cell density in liquid media, suggesting cholesterol metabolism may inhibit *M. tb* growth in some level. In culture, *M. tb* will grow on cholesterol as the sole carbon source, supplemented with asparagine, citrate and tyloxapol (*44*). Cholesterol was reported to accumulate at the site of mycobacterial entry and mediate the phagosomal association of TACO (*73*). During the infection, *M. tb* resides within macrophages and it may pass through cholesterol rich membranes of epithelial cells (*76-78*). Foamy macrophages are accumulated around granulomas in mice (*79-80*). In foamy macrophages, *M. tb* was observed within lipid bodies, and further accumulates intracellular lipids (*81*), which provide a favorable environment for persistent bacteria. Accumulation of cholesterol esters in macrophages were observed in *M. tb* (*82*), which may be responsible for the conversion of macrophages into foam cells. In human TB patients, high levels of cholesterol are found in the sputum and lipid bodies are observed (*83*). And transcriptional profiling of those specific *M. tb* showed the induction of putative genes to encode enzymes required for cholesterol utilization (*83*).

Several open reading frames in the *M. tb* genome were predicted to encode enzymes metabolizing cholesterol (*12*). Mce4 transporter (*Rv3499c-Rv3494c*), a homolog of ABC transporter, encodes a cholesterol import system and its function is essential for persistence in the mouse model (*74, 84-85*). Upon deletion of the Mce4 operon, *M. tb* grows poorly in media containing cholesterol as a sole carbon source.

Mce4 is essential for *M. tb* replication in IFN-γ stimulated macrophages and survival in mouse model of infection during late time-points, suggesting that host cholesterol import is required for chronic *M. tb* infection (*74*). Cholesterol was also reported to be converted to both CO_2 and phthiocerol dimycocerosate (PDIM) in *M. tb* by ^{14}C-labeling experiments (*74*). The CO_2 is presumably produced from acetyl CoA, generated via β-oxidation of cholesterol, passing through the glyoxalate cycle and TCA cycles. Cholesterol degradation also yields propionyl CoA, which can be converted to methyl-malonyl-CoA and therefore methyl branched lipids (*86*). Thus, the metabolism of cholesterol in *M. tb* is both anabolic and catabolic.

The *igr* operon (*Rv3540c-Rv3545c)* is transcribed as a single transcript (*87*), and the gene products were predicted to be functionally associated with the *mce4* transporter (*85*) and involved in lipid metabolism. Bioinformatic analyses predict the *igr* operon encodes a cytochrome P450, 2 acylCoA dehydrogenases, 2 conserved hypotheticals and a lipid-transfer protein (*87*). Multiple studies indicated that the *igr* locus was essential for *M. tb* growth, both in macrophages and in mice (*87-89*). Chang and coworkers have recently reported that the *igr* operon is required for cholesterol metabolism (*90*). Δigr mutant was unable to grow in the presence of cholesterol, however this phenomenon was partially reversed by a *mce4* mutant. Moreover, the *igr* phenotype during early stage of disease is completely suppressed by the *mce4* mutant, suggesting cholesterol is metabolized during *M. tb* infection (*90*). One hypothesis is that the Δigr mutant can accumulate toxic metabolites from cholesterol, which can be overcome by the disruption of cholesterol import by *mce4* deletion. The mutation of the igr locus does not affect the metabolism of the 4-carbon of cholesterol to CO_2 (*90*), indicating the *igr* genes are responsible for degradation of other portions of cholesterol. In addition, C26 is still incorporated into PDIM. Of course, the precise function in the utilization of host cholesterol by *M. tb* remains unsolved.

Proposed cholesterol metabolic pathway in M. tb

Based on sequence homology to mainly *R. RHA1*, *C. testostoroni* and *Nocardia,* the *M. tb* genes involved in cholesterol metabolism were partially mapped out. Many of genes involved in cholesterol metabolism in actinomycete genes are conserved in *M. tb* and some of them are upregulated during *M. tb* infection (*91*). The enzymes required for cholesterol A and B-ring metabolism have been characterized to varying extents (Table 1-1). However, most of the enzymes in this pathway have not been fully characterized yet.

Two ORFs including *Rv1106c* and *Rv3409c* were annotated in the *M. tb H37Rv* genome to catalyze cholesterol oxidation during mycobacterial pathogenesis. *Rv1106c* is annotated as a 3β-hydroxysteroid dehydrogenase (Hsd), and shares 74% identity with the *Nocardia* cholesterol dehydrogenase (*54*). *Rv3409c* has 24% identity and 53% similarity to the *R. equi* cholesterol oxidase (ChoD) and is annotated as a GMC oxidoreductase. Although *M. smegmatis* cellular lysates overexpressing *choD* have been reported to contain cholesterol oxidase activity (*92*), the characterization of this gene is not complete. Hsd catalyzes the first step of cholesterol metabolism in *M. tb* and is required for growth on cholesterol (Chapter 2). KstD, KshA/B, HsaC, and HsaD in *M. tb* share high levels of similarity with other actinomycete orthologs, and have been cloned and expressed in *E. coli.* The enzymatic activities of KstD and HsaD have been confirmed in cell lysates (*44, 93*). Purified KshA/B and HsaC were kinetically characterized as a 3-ketosteroid 9α-hydroxylase and an iron-dependent extradiol dioxygenase, respectively (*94-95*). In addition, the *ΔhsaC* mutant cannot grow on cholesterol as a sole carbon source. In addition, there was a reduction in granulomas in guinea pigs infected with the *ΔhsaC* mutant compared to wild-type (*94*). Enzymes metabolizing the products of HsaD are unknown.

The side chain degradation is thought to start with the oxidation of carbon 26/27 into a carboxylic acid by cytochrome P450 enzymes. However, there are 20 cyp genes in the *M. tb* genome with no identified functions available (*96*). Two highest identity homologs to ChoP in the *Streptomyces* cholesterol oxidase operon are Cyp126 (36% identity) and Cyp142 (32% identity). Cyp142 is also proposed to be cholesterol regulated (*44*). The genes involved in further side chain degradation have not been identified to date. There are 36 annotated acylCoA ligases in the *M. tb* genome. Among them, *fadD19* is part of the cholesterol regulon (*44*). Numerous sets of fatty acid β-oxidation genes have been annotated, and no essential genes have been identified yet. β-oxidation genes listed in Table 1-1 are all in the cholesterol regulon (*44*).

Table 1-1. Proposed genes involved in the cholesterol metabolic pathway.

Gene name	*M. tb*	Putative function	Studies performed	Most recent references
Hsd	*Rv1106c*	3β-hydroxysteroid dehydrogenase	B, C	In this thesis, (*97*)
kstD	*Rv3537*	ketosteroid Δ1-dehydrogenase	B	(*93*)
kshA	*Rv3526*	3-Ketosteroid 9α-hydroxylase	B	(*95*)
kshB	*Rv3571*	3-Ketosteroid 9α-hydroxylase	B	(*95*)
hsaA	*Rv3570c*	3-HSA-hydroxylase	A, D	(*44*)
hsaB	*Rv3567c*	3-HSA-hydroxylase	A	(*44*)
hsaC	*Rv3568c*	2,3-dehydroxyphenyl dioxygenase	B, C	(*44, 94*)
hsaD	*Rv3569c*	4,9-DSHA hydrolase	A	(*44*)
cyp125	*Rv3545c*	Cytochrome P450	A	(*44*)
fadD19	*Rv3515c*	acylCoA lygase	A,D	(*44*)
echA19	*Rv3516*	enoylCoA hydratase	A,D	(*44*)
fadA5	*Rv3546*	thiolase	A,D	(*44*)
fadE29	*Rv3543c*	acylCoA dehydrogenase	A,D	(*44*)
choD	*Rv3409c*	GMC oxidoreductase	A	(*92*)
cyp142	*Rv3518c*	Cytochrome P450	A	(*44*)

A. Bioinformatic annotation
B. Recombinant expression and enzymatic function confirmed
C. Function confirmed with mutant strain *in vivo*
D. Function examined with mutant strain *in vivo* (in this thesis) (*87, 90, 98*)

III. Hydroxysteroid dehydrogenases

Overview of the short-chain dehydrogenases/Reductases (SDR) superfamily

The short-chain dehydrogenase/reductase superfamily is a large group of functionally heterogeneous enzymes in different domains of life (*99*). To date, over 3000

members of SDRs have been found in protein or nucleic acid databases (*100*). Although the sequence identity of this family is only around 15-30% (*101*), the N-terminal region is structurally conserved and binds to NAD(P)(H) as a cofactor. From the available 3D structures of this superfamily, the most conserved feature is a classical Rossmann-fold scaffold for nucleotide binding, i.e., an α/β folding pattern with a central β sheet surrounded by α-helices, 2-3 on each side. Contrary to the N-terminal, the C-terminal of this protein family is variable, which enables SDRs to perform oxidoreductase, lyase and isomerase reactions and accommodate a wide spectrum of substrates including steroids, lipids, amino acids, carbohydrates, hormone, and xenobiotic compounds (*102-103*).

Using the Hidden Markov model (HMM) analysis, the SDR superfamily were clustered into five additional families: two main types, denoted 'classical' and 'extended' (*104*), and three further types, denoted 'intermediate', 'complex', and 'divergent' (*105*). The five families of proteins can be distinguished based on their characteristic sequence motifs of cofactor binding sequence and active site (Table 1-2). Cofactor NAD(H) and NADP(H) bind to the Rossmann fold part of the enzymes, the structural motif of which is characterized by a highly variable Gly-rich sequence pattern. NAD(H) prefers enzymes containing an acidic residue forming hydrogen bonds to the 2' and 3'-hydroxyl groups of the adenine ribose, located at C-terminal end of the second core β-strand (*106*). NADP(H) prefers enzymes containing two basic residues, one is located after the crucial acidic residue of NAD(H)-preferring enzyme, which compliment the additional phosphate group of NADP(H), and another basic residue resides in the Gly-rich motif before the second glycine (*107-108*). Statistically, classical SDRs show an overall preference for NADP(H) and extended SDRs show preference for NAD(H) (*109*).

Table 1-2. Conserved sequence motifs in SDRs.

Cluster	Cofactor binding	Active site	Typical enzymes
classical	TGxxxA/GxG	YxxxK	oxidoreductases
extended	S/TGxxGxxG	YxxxK	isomerases, lyases, 3βHSD proteins
intermediate	G/AxxGxxG/A	YxxxK	alcohol dehydrogenases
complex	GxxxxxSxA	YxxMxxxK	fatty-acid synthases, polyketide synthases
divergent	GGxGxxG	YxxxN	enoyl-thioester reductases

The common mechanism of SDRs lies in a hydride and proton transfer involving the nicotinamide. The typical acid-base catalysis in SDRs utilizes a hydroxyl-tyrosinate ion that donates or abstracts a proton to or from the substrate (*110*), and a downstream Lys residue lowers the tyrosine hydroxyl pKa, together with cofactor nicotinamide. This mechanism is based on the active motif Gly-x-x-x-Lys. However, the 'divergent' and 'complex' SDRs utilize distinct mechanisms (Table 1-2).

Hydroxysteroid dehydrogenases: intriguing drug targets for metabolic syndromes

Hydroxysteroid dehydrogenases (Hsd), which convert hydroxyl into keto-groups or vice versa at key positions of steroids, belong to the SDR superfamily, including mainly 3βHSD, 11βHSD, 17βHSD (*101, 111*), or the aldo-keto reductase (AKR) superfamily, including 3αHSD, 20αHSD and Type 5 17βHSD (*112-113*). SDRs contain a typical "Rossman fold" within the N-terminal (vide supra) to accommodate the nicotinamide cofactor. However, the AKRs share a TIM barrel fold with an $(\alpha/\beta)_8$ pattern, where each β-strand alternates with an α-helix running anti-parallel to the strand, functioning as a binding pocket to nicotinamide cofactor (*114*), and vary in loops on the C-terminal side of the barrel for substrate specificity.

Functionally, HSDs are essential for the biosynthesis or metabolism of all steroid hormones (*115*), despite the dissimilar structures of this subfamily. All HSDs require nicotinamide cofactors to catalyze the hydride transfer-oxidation or reduction, which is

fundamentally a reversible process that can be regulated by a thermodynamic equilibrium constant. However, the directional preference of an individual HSD does not always follow the free energy direction, i.e., NAD(P)H to $NAD(P)^+$. Although the intracellular cofactor regulation is not fully clarified, the intracellular redox state, as well as nicotinamide cofactor ratio and preference, are the driving force of HSD reactions (*116-117*).

Many HSDs are potential therapeutic targets. 17βHSD catalyzes the final steps of specific steroidal hormones, such as androgen and estrogen, biosynthesis. 11βHSDs are involved in glucocorticoid regulation through the conversion of inactive form (cortisone in human, dehydrocorticosterone in rodents) to active form (cortisol in human, corticosterone in rodents). Two isozymes (11βHSD1 and 11βHSD2) have been identified. Inhibitors to 11βHSD1 target metabolic syndromes, such as insulin resistance, hyperlipidemia and obesity (*118-119*), and inhibitors to 17βHSD are targets for sex steroid metabolism, e.g. in breast and prostate cancer treatment (*120*).

3β-hydroxysteroid dehydrogenase

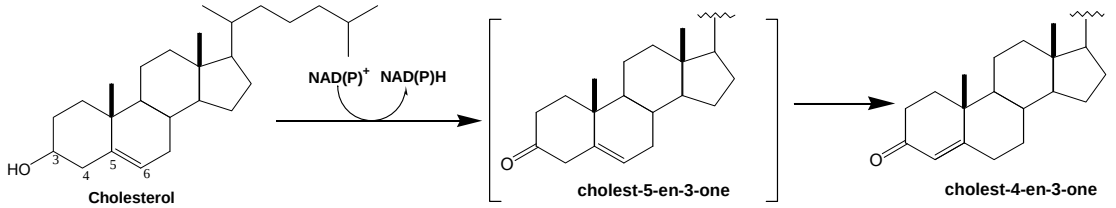
The enzyme 3β-hydroxysteroid dehydrogenase (3βHSD) catalyzes the oxidation and isomerization of 3β-hydroxysteroids to Δ^4-3-ketosteroids (*121*) (Scheme 1-4), which is an essential step for the eukaryotic biosynthesis of all classes of active steroids: progesterone, mineralocorticoids, glucocorticoids, androgens, and estrogens (*122*). 3βHSD in *vaccinia virus* has been identified to correlate with the production of corticosterone in the infected host (*123*). A Δ3βHSD mutant strain of *vaccinia virus* was attenuated and a reduced cortisterone level in lungs and plasma of infected animal models was observed. Other factors IFNγ, $CD4^+$, and $CD8^+$ showed enhanced production in the Δ3βHSD mutant strain. The data provided evidence that the enzyme affected virus virulence by immunosuppression (*123*).

Multiple isoforms of 3βHSD exist in human, rat and mouse tissues. In mammalian cells, two isomers of 3βHSD are found. $NAD(P)^+$ is a cofactor to this enzyme and the coenzyme product, NADPH activates the isomerase reaction by generating a conformational change (*124*). Furthermore, kinetic analyses elucidated that the 3β-hydroxysteroid dehydrogenase and isomerase activities share the same coenzyme domain in human 3βHSD (*125*). 3βHSD is also a potent drug target. A combination of a 3βHSD1 inhibitor and an aromatase inhibitor are used in the treatment of breast cancer (*126*).

Computer analyses revealed that *Norcardia* 3βHSD (previously named as cholesterol dehydrogenase) and mammalian 3βHSD are homologs not only in sequences but also in functions (*54, 125*). Cholesterol dehydrogenase has been isolated from some microorganisms, such as *Flammulina sp.* and *Trametes sp* (*127*). *Nocardia* sp. strain Ch 2-1 was discovered to be a $NAD(P)^+$-dependent cholesterol dehydrogenase producing bacteria, by screening for microorganisms which produce $NAD(P)^+$-dependent cholesterol dehydrogenase. *Nocardia* cholesterol dehydrogenase is transcriptionally regulated by the addition of cholesterol to culture medium. This cholesterol dehydrogenase gene has been cloned and expressed in *Streptomyces lividans* and the recombinant enzyme showed a substrate preference for 3β-hydroxysteroids containing a side chain at C17 position, such as cholesterol and β-sitosterol, and ergosterol (*54, 128*).

One application of cholesterol dehydrogenase is to measure serum cholesterol concentrations in the diagnosis and treatment of arteriosclerosis. This serum cholesterol assay includes two enzymes: cholesterol dehydrogenase and cholesterol ester hydrolase. Serum cholesterol esters are first hydrolyzed to free cholesterol by cholesterol ester hydrolase and the free cholesterols are then reduced into cholest-4-en-3-one by cholesterol dehydrogenase. Cholest-4-en-3-one in the reaction is directly

detected by measuring the absorbance of NAD(P)H at 340 nm (*129*). Cholesterol dehydrogenase from *Nocardia* sp was recommended for this assay.



Scheme 1-4. 3βHSD catalyzes the oxidation and isomerization of cholesterol to cholest-4-en-3-one.

As discussed above, *Rv1106c* encoding 3βHSD in *M. tb* is 74% identical to *Nocardia* cholesterol dehydrogenase and is 29% identical to human 3βHSD. The characterization of *M. tb* 3βHSD has not been studied previously. This thesis describes the characterization of *M. tb* 3βHSD and the identification of several enzymes and the metabolites involved in this pathway. Understanding of cholesterol metabolism and its relationship to *M. tb* virulence will ultimately help finding new drug targets against TB and understanding *M. tb* persistence in the host.

IV. References

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Chapter 2

3β-Hydroxysteroid Dehydrogenase encoded by *Rv1106c* from *Mycobacterium tuberculosis*

This chapter is adapted from a paper that was published in *Biochemistry* (1).

I. Introduction

Tuberculosis is an opportunistic infection caused by *Mycobacterium tuberculosis* (*M. tb*) in individuals with HIV-AIDS that is estimated to infect 30% of the world's population (*2-3*). The World Health Organization estimates that 2 million people die every year from tuberculosis. Drug resistance to front-line *M. tb* drugs rifampicin and isoniazid has emerged (*4-5*) and additional resistance to second line drugs is emerging (*6-7*). It is clear that new approaches are required to combat these multi drug-resistant and extreme (or extensively) drug-resistant organisms (*8-10*).

Rv1106c is annotated as a cholesterol dehydrogenase because it is 74% identical with the *Nocardia sp.* cholesterol dehydrogenase. Cholesterol dehydrogenase is an NAD^+ or $NADP^+$ dependent enzyme and catalyzes two sequential reactions: oxidation of cholesterol (3β-hydroxysterol) to cholest-5-en-3-one and isomerization of cholest-5-en-3-one to cholest-4-en-3-one. The expression of *Nocardia* cholesterol dehydrogenase is reported to be transcriptionally activated by the addition of cholesterol to culture medium (*11-12*). The *M. marinum* (pathogen that causes fish and amphibian tuberculosis) homolog is preferentially expressed in the frog granuloma (*13*). Thus, it appears that transcription and expression may be cholesterol dependent, and may require the host to provide the sterol activator.

Gene database analysis has revealed a superfamily of proteins that in addition to the bacterial cholesterol dehydrogenase includes mammalian 3β-hydroxysteroid dehydrogenases, plant dihydroflavonol reductases, bacterial UDP-galactose-4-epimerases and viral 3β-hydroxysteroid dehydrogenases (*14-15*) (Figure 2-1). There appears to be an ancient evolutionary relationship between plant flavonol synthesis and mammalian steroid hormone synthesis and in fact, many flavonols are agonists or antagonists of mammalian steroid hormone receptors (*16*). The viral 3β-hydroxysteroid dehydrogenases are genetically closest to the mammalian 3β-hydroxysteroid

dehydrogenases and the vaccinia virus dehydrogenase functions in viral steroid hormone synthesis. A mutant strain of vaccinia virus carrying a knockout deletion of the vaccinia gene coding for 3β-hydroxysteroid dehydrogenase is attenuated in mice (*17*). Unlike the wild-type virus, the mutant does not induce the formation of corticosterone, a suppressor of the host inflammatory response to infection. The bacterial 3β-hydroxysteroid dehydrogenases are more distantly related to the mammalian dehydrogenases than their viral counterparts (Figure 2-1), but they may serve a function similar to the viral enzymes, that is, steroid hormone synthesis, or they may be involved in bacterial flavonol synthesis. Both activities could lead to immune suppression. The ability of actinomycetes such as *M. tb* or *Nocardia*, to persist in the intracellular milieu requires mechanisms for abrogating the normal host immune response and targeting the glucocorticoid receptors may be one such mechanism.

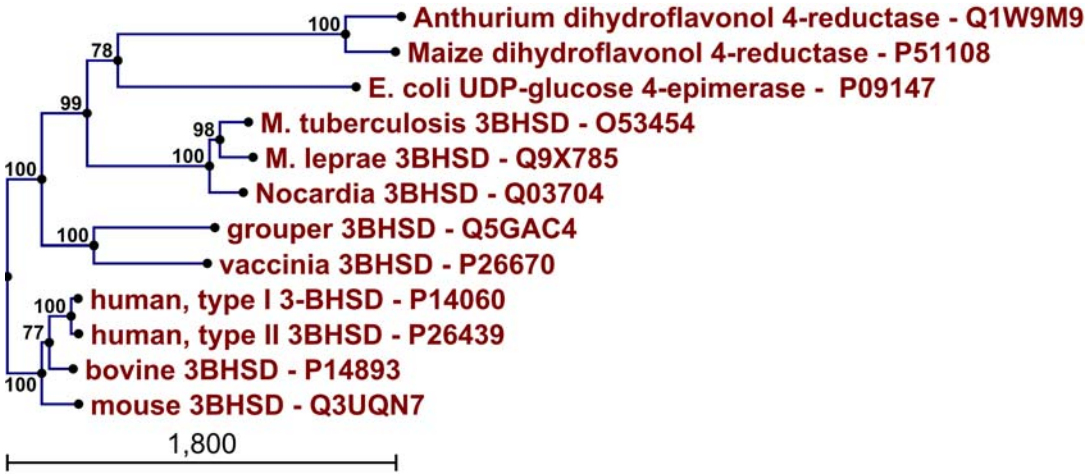


Figure 2-1. Unrooted phylogenetic tree for 3β-hydroxysteroid dehydrogenase encoded by *Rv1106c* and related genes. The values are relative evolutionary distance. The tree was generated using a ClustalW 1.82 alignment and CLC Free Workbench 3. Proteins are identified by species, enzyme, and Uniprot entry. [reprinted with permission from (1); copyright 2007, American Chemical Society].

The similarities among the enzymes of this superfamily hint at the potential catalytic properties of *Rv1106c* from *M. tb*. However, the physico-chemical characteristics of this putative 3β-hydroxysteroid dehydrogenase from *M. tb* have not been defined and its real

enzymatic function has not yet been confirmed. Before assigning physiological function, we undertook a fundamental characterization of the chemistry catalyzed by this putative 3β-hydroxysteroid dehydrogenase encoded by *Rv1106c* and the results of those studies are presented here.

II. Experimental procedures

Materials and general methods. Cholesterol, cholest-4-en-3-one, cholest-5-en-3-one, NAD^+, NADH, 5-pregnen-3β-ol-20-one, trilostane, and Tween-80 were purchased from Sigma-Aldrich (St. Louis, MO). Triton X-100 and progesterone were from Aldrich Fine Chemical Co. (Milwaukee, WI). $NADP^+$ was from Research Products International Co. (Mount Prospect, IL). Restriction endonucleases and T4 DNA ligase were from New England Biolabs (Beverly, MA). Oligonucleotides were from IDT Inc. (Coralville, IA). Cloned pfu DNA polymerase was from Stratagene (La Jolla, CA). Total genomic DNA of *M. tuberculosis* H37Rv was obtained from the TB Research Materials Facility at Colorado State University (Fort Collins, CO) (NIAD NO1-AI40091). MALDI mass spectra were acquired on a Bruker Autoflex II TOF/TOF. Cloned Pfu DNA polymerase was from Stratagene (La Jolla, CA). Big Dye DNA sequencing (Applied Biosystems, Foster City, CA; performed by the Stony Brook University Sequencing Facility) was used to verify the coding sequence of the expression plasmids. DEAE-52 cellulose was from Whatman (Maidstone, UK). Butyl sepharose 4B, Q-sepharose, blue sepharose 6 fast flow and sephacryl S-200 (high resolution) was from GE Healthcare Biosciences Corp. (Piscataway, NJ). All other chemicals and solvents of reagent grade were supplied by Fisher Scientific (Pittsburgh, PA). 7H9 standard medium is composed of Middlebrook 7H9 medium supplemented with 10% albumin–dextrose saline (10×ADS: 8% NaCl, 10% BSA fractionV, 4% glucose), 0.2% glycerol, and 0.05% Tween-80. 7H9 expression medium is Middlebrook 7H9 medium supplemented with 0.05% Tween-80, 0.2% glucose

and 0.2% glycerol. 2xYT is composed of 16 g tryptone, 10 g yeast extract and 5 g NaCl per liter. Buffers used were, A: 50 mM triethanolamine hydrochloride, pH 8.5; B: buffer A + 0.2 mM NaCl; C: 100 mM triethanolamine hydrochloride buffer, pH 8.5; D: buffer C + 0.05% (w/v) triton X-100; E: 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5; F: buffer E + 0.05% triton X-100.

Construction of Mycobacterium smegmatis expression plasmids pFPCAP-1106c, pSD26-1106c, pSD31-1106c, pSD26-1106c-2 and pVV16-1106c. The *Rv1106c* gene was amplified from *M. tuberculosis* H37Rv total genomic DNA by PCR using primer 1 and primer 2. The PCR product was digested with BamHI and EcoRI and ligated into a similarly digested pFPCAP1 vector (*18*) to create *M. smegmatis* expression vector pFPCAP-1106c. The *Rv1106c* gene was then subcloned from pFPCAP-1106c by PCR using primer 1 and primer 3. This amplified fragment was digested with BamHI and EcoRV and ligated into similarly digested pSD26 to create plasmid pSD26-1106c or ligated into similarly digested pSD31 that contained a “Myco-N-His” epitope tag to create plasmid pSD31-1106c. In pSD26-1106c, the “Myco-C-His” epitope tag of vector pSD26 was removed during the PCR amplification and a stop codon inserted directly after the last native codon of the *Rv1106c* gene. The *Rv1106c* gene was subcloned from pFPCAP-1106c by PCR using primer 1 and primer 4. This amplified fragment was then digested with BamHI and EcoRV into similarly digested pSD26 that contained a “Myco-C-His” epitope tag to create plasmid pSD26-1106c-2. The *Rv1106c* gene was subcloned from pFPCAP-1106c by PCR using primer 5 and primer 6. This amplified fragment was then digested with NdeI and HindIII and ligated into similarly digested pVV16 that contained a “Myco-C-His” epitope tag to create plasmid pVV16-1106c. DNA sequencing of the plasmids confirmed that the full *RV1106c* sequence was inserted correctly and that no mutations were introduced during the cloning procedures. All plasmids were introduced into *M. smegmatis* mc^2155 by electroporation.

Table 2-1. Primers and restriction sites used for construction of 3β-hydroxysteroid dehydrogenase from *M. tb* and mutants.

Primer[#]	Primer code	Restriction site	Sequence [a,b]
1	ChoDH F BamHI	BamHI	5'-gagGGATCCatgcttcgccgcatgggtg-3'
2	ChoDH R 6xHis EcoRI	EcoRI	5'-gtgCAATTCcta**gtggtggtggtggtggtg**cggcttgactgtggcggccg-3'
3	ChoDH R EcoRV	EcoRV	5'-ggtgGATATCctacggcttgactgtggcggccg-3'
4	ChoDH R EcoRV 2	EcoRV	5'-ggtgGATATCcggcttgactgtggcggccg-3'
5	ChoDH F NdeI	NdeI	5'-gagCATATGcttcgccgcatgggtg-3'
6	ChoDH R HindIII	HindIII	5'-ggtgAAGCT Tcggcttgactgtggcggccg-3'
7	ChoDH R HindIII+	HindIII	5'-ggtgAAGCT Tctacggcttgactgtggcggccg-3'

[a] The restriction sites are denoted with capital letters, and the C-terminal six-histidine tag added by PCR is shown in boldface type.

Primer[#]	Mutation site	Code	Restriction site	Sequence [b]
8	N109Q	KR1	NdeI	Forward: 5'-gcccgtagctttgcggtccaggtcggcggcaccgagggc-3'
		KR2	HindIII	Reverse: 5'-gccctcggtgccgccgacctggaccgcaaagctacgggc-3'
9	S131A	KR3	NdeI	Forward: 5'-gcccggttcgtctacacgtcagccaccagtgtggtgatgggc-3'
		KR4	HindIII	Reverse: 5'-gcccatcaccacactggtggctgacgtgtagacgaaccgggc-3'
10	Y158F	KR5	NdeI	Forward: 5'-gcccggttcaacgacctcttcaccgagaccaaggtggttgcc-3'
		KR6	HindIII	Reverse: 5'-ggcaaccaccttggtctcggtgaagaggtcgttgaaccgggc-3'
11	K162I	KR7	NdeI	Forward: 5'-gccctctacaccgagaccatcgtggttgccgagcgattc -3'
		KR8	HindIII	Reverse: 5'-cgcgaatcgctcggcaaccacgatggtctcggtgtagagggc-3'

[b] The mutated bases are denoted with underlined letters.

Construction of E. coli Expression Plasmids pET20b-1106c, pET28b-1106c and pET29a-1106c. The *Rv1106c* gene was subcloned from pFPCAP-1106c by PCR using primer 5 and primer 6 and ligated into similarly digested pET20b that contained a C-terminal His_6-tag to create plasmid pET20b-1106c. The *Rv1106c* gene was subcloned from pFPCAP-1106c by PCR using primer 5 and primer 7. The amplified fragment was digested with NdeI and HindIII and ligated into similarly digested pET28b that contained an N-terminal His_6-tag of the plasmid to create plasmid pET28b-1106c or ligated into similarly digested pET29a that did not contain an N-terminal His_6-tag. The C-terminal His_6-tag of pSD26 was removed during the PCR amplification and a stop codon inserted directly after the last native codon of the *Rv1106c* gene. DNA sequencing of the plasmids confirmed that the full *RV1106c* sequence was inserted correctly and that no mutations were introduced during the cloning procedures. All plasmids were transformed into *E. coli* BL21(DE3)*pLysS.*

Expression of the Rv1106c gene products in M. smegmatis. M. smegmatis mc^2155 carrying one of the various expression plasmids was cultured for one to two days in 7H9 standard medium with appropriate antibiotics. Starter cultures containing plasmids with the inducible acetamidase promoter were used to inoculate 7H9 expression medium with appropriate antibiotics at a ratio of 1:50, and these expression cultures were grown for 15h. When they reached an OD_{600} = 0.5~0.6, the culture was induced with 0.2% (w/v) acetamide and grown for another 12–16 h. The cells were collected by centrifugation at 13,000 g for 30 min. *M. smegmatis* cells were suspended in buffer A, and lysed with a French press at 10,000 psi. Cell debris was removed by centrifugation at 135,000g for 3 h.

Expression and purification of the Rv1106c gene product in E. coli. BL21(DE3)*pLysS* transformed with pET28b-1106c or pET29a-1106c was cultured overnight in LB/kanamycin medium. Starter cultures were used to inoculate 2xYT

medium (30 μg/mL kanamycin) at a ratio of 1:100 for around 3 h until OD_{600}=0.6~0.8. Isopropyl β-D-thiogalactoside (IPTG) was added at a final concentration of 0.4 mM to induce protein expression. Cultures were grown at 25 °C for an additional 8 h or at 18 °C for an additional 20 h. The cells were collected by centrifugation at 5,000 g for 30 min. The cell pellet (5 g) was suspended in 30 mL of buffer A and lysed by French press at 10,000 psi. All subsequent steps were conducted at 4°C. Cell debris was removed by centrifugation at 135,000 g for 3 h. The supernatant was precipitated with ammonium sulfate and the 5-10 % (w/v) fraction was collected. This pellet was suspended in buffer A (5 mL) and dialyzed (NMWCO 6,000 - 8,000) against buffer A. The dialysate was loaded onto a column of DEAE-cellulose (30 mm × 25 cm, DE 52) equilibrated with buffer A. Fractions were collected by gradient elution (0.1 to 0.4 M NaCl) and 10 mL fractions were collected. Fractions with activity were combined, concentrated and desalted by ultrafiltration (NMWCO 10,000) to a final volume of 5 mL. This active fraction was then applied to Q-Sepharose (10 mL bed volume) equilibrated in buffer B, and the flow-through was collected and concentrated to a final volume of 2 mL. The concentrate was loaded on a Sephacryl S-200 high-resolution column (20 mm × 120 cm) and eluted with buffer B. Pure fractions containing dehydrogenase activity were collected and concentrated.

The identity of the purified protein was confirmed by tryptic digestion and MALDI mass fingerprinting. Dehydrogenase (~5 μg in gel) was digested with trypsin (400 ng) in 20 μL 50 mM NH_4HCO_3 for 17 h. The tryptic fragments were extracted with 60% CH_3CN/H_2O, 0.1% TFA (50 μL) three times. The combined extracts were dried and redissolved in 5 μL 0.1% TFA/H_2O. MALDI-TOF mass spectra were acquired in positive ion mode using a saturated solution of α-cyano-4-hydroxy cinnamic acid as the matrix.

Activity assays. Initially, the 3β-hydroxysteroid dehydrogenase was assayed at 30 °C for less than 10% of reaction with monitoring of NADH absorbance at 340 nm in buffer C (pregnenolone, DHEA, quercetin or dihydroquercetin as substrate) or buffer D (cholesterol as substrate). Dehydroepiandrosterone, pregnenolone, quercetin or dihydroquercetin stock solutions were prepared in ethanol (3 mM), and cholesterol stocks were prepared in propan-2-ol. The final volume of alcohol was kept constant in all assays and was 5%. Reactions were initiated by the addition of dehydrogenase. Later activity measurements were made as described below for enzyme kinetics.

Product analysis by TLC and HPLC spectroscopy. Enzyme assay solutions (1 mL) at various extents of reaction were extracted with an equal volume of ethyl acetate five times. The ethyl acetate extracts were combined and dried under a stream of N_2. Then the dried residue was dissolved in 50 µL ethyl acetate and applied to silica gel thin layer chromatography plates and separated using hexane:ethyl acetate, 4:1 or dichloromethane:ethyl acetate, 5:1. To improve resolution, the plate was developed three times drying the plate in between each run. Steroid products were visualized by UV and staining with phosphomolybdic acid in ethanolic solution (10% w/v). Authentic standards were run on the same plate and co-spotted with the reaction mixtures.

Samples for HPLC analyses (*19*) were analyzed on a Shimadzu Corp. system (Columbia, MD) comprised of a SCL-10A VP system controller, two LC-10AD solvent pumps, and a Model SPA-20A Prominence UV/VIS. The following conditions were used: stationary phase, Microsorb-MV® C-18 column (Rainin Instrument Corp., Woburn, MA, 5 µm, 100 Å, 4.6 × 250 mm); mobile phase, solvent A, CH_3CN/H_2O (90:10, v:v) and solvent B, CH_3CN/propan-2-ol (85:15, v:v). Samples were eluted for 25 min isocratically with solvent A, followed by a 10-min linear gradient to 100% solvent B, followed by a 45-min isocratic elution with solvent B; the flow rate was 1.25 mL/min; peaks were detected at 212 and 240 nm. Sample aliquots (200 µL) were injected directly from enzyme assay

solutions incubated at 30°C that contained 120 μM cholesterol, 5% iso-propanol, 1.4 mM NAD and 0.41 pM dehydrogenase in buffer D. Calibration curves for cholesterol and cholest-5-en-3-one were prepared by the integration of peak area detected at 212 nm for standard solutions ranging in concentration from 0 to 1500 μM. Calibration curves for cholest-4-en-3-one were prepared analogously by the integration of peak area detected at 212 and 240 nm.

Enzyme kinetics. The 3β-hydroxysteroid dehydrogenase was assayed at 30 °C for less than 10% of reaction with monitoring of NADH absorbance at 340 nm in buffer E (pregnenolone or DHEA as substrate) or buffer F (cholesterol as substrate). DHEA or pregnenolone stock solutions were prepared in ethanol (3 mM) and cholesterol stocks were prepared in propan-2-ol. No oxidation of ethanol or propanol was detected in control assays. The enzymatic activity was insensitive to alcohol concentrations between 2.5% and 5% alcohol, and the final volume of alcohol was kept constant in all assays and was 5%. Reactions were initiated by the addition of dehydrogenase. Initial velocities were measured for varying NAD^+ concentrations (350 - 22400 μM) at different fixed concentrations of DHEA or pregnenolone (30 - 300 μM). Initial velocities were measured for varying trilostane concentrations (0 - 1000 μM) at different fixed concentrations of NAD^+ (88 - 5600 μM) with DHEA at a concentration of 120 μM or at different fixed concentrations of DHEA (11 - 240 μM) with NAD^+ at a concentration of 350 μM. All points are the average of triplicate data points.

Initial velocities were globally fit to the following equations using Grafit software (Version 4.0.10).

Ternary complex Michaelis-Menten equation

$$v = V_m[A][B]/\{K_{ia}K_{mb} + K_{mb}[A] + K_{ma}[B] + [A][B]\} \quad \text{(Eq. 1)}$$

Competitive inhibition equation

$$v = V_m[S]/\{K_m(1+[I]/K_{ic}) + [S]\} \quad \text{(Eq. 2)}$$

Uncompetitive inhibition equation

$$v = V_m[S]/\{K_m + [S](1+[I]/K_{iu})\} \quad \text{(Eq. 3)}$$

Mixed inhibition equation

$$v = V_m[S]/\{K_m(1+[I]/K_{ic}) + [S](1+[I]/K_{iu})\} \quad \text{(Eq. 4)}$$

Substrate NAD^+ inhibition equation

$$v = V_m[NAD^+]/(K_m + [NAD^+] + [NAD^+]^2/K_{iuNAD+}) \quad \text{(Eq. 5)}$$

Fixed second substrate Michaelis-Menten equation

$$v = V_m[S]/(K_m + [S]) \quad \text{(Eq. 6)}$$

Rate pH dependence equation

$$\text{rate} = \text{rate}_m 10^{(pH-pKa)}/\{10^{(pH-pKa)}+1\} \quad \text{(Eq. 7)}$$

where K_{ma} and K_{mb} are the Michaelis constants for A and B at saturating concentrations of B and A, respectively, K_m is the apparent Michaelis constant for a varied substrate at a fixed concentration of the second substrate. K_{ia} is the dissociation constant for enzyme and A, V_m is the maximum velocity, S is the varied substrate, K_{ic} and K_{iu} are the competitive and uncompetitive inhibition constants, respectively, rate is either k_{cat} or k_{cat}/K_m and rate_m is the maximal value of either k_{cat} or k_{cat}/K_m.

The pH dependence of the 3β-hydroxysteroid dehydrogenase-catalyzed reaction was determined in 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$ and 2.8 mM NAD^+ at different fixed concentrations of DHEA (11 - 240 μM) between pH 6.5 and 9.0. Initial velocity data were fit to eq. (6) to determine the apparent k_{cat} and k_{cat}/K_m values at each pH. The apparent pK_a's were then determined from these rate constants and eq. (7).

Culture of CDC1551 and Rv1106c expression analysis. The *M. tb* strains used in this study were derivatives of *M. tb* CDC1551. The *Mt1137* ORF corresponds to the *Rv1106c* ORF in *M. tb* H37Rv. The *Mt1137* transposon mutant (*20*) was obtained through TARGET, part of NIAID contract #HHSN266200400091C for Tuberculosis

Vaccine Testing and Research Materials awarded to Colorado State University. The *M. tb* cells were cultured at 37 °C in Middlebrook 7H9 (liquid) or 7H10 (solid) (Difco) media that were supplemented with 10% albumin-dextrose-sodium chloride complex (ADS) (*21*), 0.2% glycerol and 0.05% Tween 80. The kanamycin concentration used for the transposon mutant in *M. tb* was 20 μg/mL. Induction with cholesterol was done by addition of cholesterol (1 mg/mL final concentration) solubilized in Tween-80 (5% w/v final concentration) to cultures at the mid-logarithmic phase. Cultures were incubated for 24 h at 37 °C and harvested by centrifugation. The culture filtrate was collected and sterile filtered for HPLC assay and enzymatic analysis. The cell pellet was washed by resuspension in 1 mL 50 mM sodium phosphate buffer, pH 7 containing 5% v/v propan-2-ol, the cells pelleted again, and the process repeated two more times to remove cholesterol from the medium. The pellet was resuspended in the same buffer and the cells lysed by bead-beating twice for 1 min, with incubation on ice for 2 min between each bead beating. The cell lysate was centrifuged to remove the cell debris and the supernatant was sterilized by filtration and analyzed for enzymatic activity by HPLC assay. Lysates were incubated with 150 μM cholesterol and 3.5 mM NAD^+ in buffer D at 30 °C for 72 h and then directly injected onto a C18 reversed-phase HPLC column for analysis as described above. A unit of cholesterol oxidation activity was defined as 1 μmol cholest-4-en-3-one/min•mg protein under these assay conditions.

Site-directed mutagenesis, expression, purification and kinetic studies of mutant enzymes. Mutations of S131A, Y158F, K162I and N107Q were introduced by PCR mutagenesis using primers 8-11 (Table 2-1). DNA fragments with the targeted mutation sites digested by NdeI and HindIII was ligated into pET28a vector, which was digested by the same restriction enzymes, with T4 DNA ligase. All of the resulting mutant genes were verified by dideoxy DNA sequencing. This work was performed by Kirthana Raman and Dr. Natasha M. Nesbitt. These mutant 3β-hydroxysteroid dehydrogenases

were expressed and purified as described above for pET28b-1106c. Kinetic constants for sterol substrate were determined for wild-type and mutant enzymes with a fixed concentration of NAD^+ at 1.4 mM and dehydroepiandrosterone concentrations from 3 μM to 150 μM. And initial velocity data were fit to eq. (6) to determine the apparent k_{cat} and K_m values.

Other analyses. The primary sequences of proteins related to *Rv1106c* were obtained from a BLASTP search initiated on the NIH server (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple-sequence alignments and the phylogenetic tree were produced by the Protein Information Resource (*22*) using CLUSTALW 1.82 (*23*) and CLC free workbench 3.2 with default parameters. Signal peptide analysis was performed on the Center for Biological Sequence Analysis server (http://www.cbs.dtu.dk/services/) using SignalP 3.0 (*24*) and Secretome 2.0 (*25*). Operon prediction was analyzed on The Institute for Genomic Research website (http://www.tigr.org/tigr-scripts/operons/operons.cgi) (*26*).

III. Results and discussion

Bioinformatics analysis of Rv1106c. Protein family analysis indicated that *Rv1106c* is most closely related to the *Nocardia* species cholesterol dehydrogenase (75% identity, UniProtKB ID Q03704) and other mycobacterial homologs (Fig 2-1). It shares 24% amino acid identity with the vaccinia virus 3β-hydroxysteroid dehydrogenase (UniProtKB ID O57245) and 29% identity with the human ortholog (UniProtKB ID P14060).

In the *M. tb* H37Rv genome, the *Rv1106c* gene is located within 10 bases of the *xseB* (*Rv1107c)* and *xseA* (*Rv1108c*) genes, probable exonuclease VII small and large subunits, and 18 bases of a possible nitrobenzyl esterase (*Rv1105c*). Similar gene pairs are found only in mycobacterial genomes, e.g., *Mycobacterium bovis*, *Mycobacterium*

avium paratuberculosis, and *Mycobacterium leprae*. The *Nocardia farcinica* 3β-hydroxysteroid dehydrogenase homolog *nfa34580* is not paired with the exonuclease VII subunits. Signal peptide analysis indicated that the probability that the *Rv1106c* gene product is secreted classically with a signal peptide or nonclassically is less than 0.5 and 5%, respectively.

The *Rv1106c* gene product appears to be a member of the short-chain alcohol dehydrogenase superfamily (*27*). The glycine-rich sequence Gly-X-X-Gly-X-X-Gly, synonymous to the Rossman fold sequence Gly-X-Gly-X-X-Gly for AMP binding is present as Gly^{21}-Gly-Gly-Ala-Gly-Phe-Val-Gly^{27}. The same Gly-X-X-Gly-X-X-Gly variation of the motif is present in mammalian 3β-hydroxysteroid dehydrogenases (*28*) (Figure 2-9). Moreover, the short-chain alcohol dehydrogenase active site motif Tyr-X-X-X-Lys appears as Tyr^{158}-Thr-Glu-Thr-Lys^{162} in the translated *Rv1106* gene (Figure 2-9). Lastly, a single aspartate (Asp^{36} in the human type 1 3β-hydroxysteroid dehydrogenase) thought to form hydrogen bonds with the 2', 3' hydroxyls of the cofactor ribose dictates dehydrogenase specificity for NAD^+ rather than $NADP^+$ (*29*). This aspartate is conserved in the *Rv1106c* sequence (Asp^{45}) suggesting that the *Rv1106c* gene product will preferentially use NAD^+ as a cofactor.

Recombinant expression studies. Attempts to express the *Rv1106c* gene in *M. smegmatis* behind the acetamidase promoter were unsuccessful. *Rv1106c* was expressed in *E. coli* BL21(DE3)plysS with an N-terminal His_6 tag. The expressed protein was present in both the soluble and the membrane fractions. The soluble expressed protein did not bind to Ni^{2+} or Co^{2+} IMAC columns in its native form, suggesting that the N-terminus is not exposed in the folded protein. However, it could be purified by IMAC under denaturing conditions (6M guanidium hydrochloride), thus confirming that the expressed protein contained an N-terminal his-tag. The protein identity was confirmed by MALDI-TOF mass analysis of a tryptic peptide mixture generated by in-gel digestion.

Eighteen percent of the sequence was covered (Table 2-2). Expression levels in *E. coli* BL21(DE3)plysS were 10-fold lower without the N-terminal fusion or with only the C-terminal fusion. The expressed proteins with and without the N-terminal His_6 tag were purified by conventional methods to yield $rH_6$3βHSD and r3βHSD, respectively. The apparent MW was determined by SDS-PAGE to be 43 kD, as expected for a protein that is not post-translationally processed. On a native gel, the apparent MW of the recombinant protein was that of a monomer.

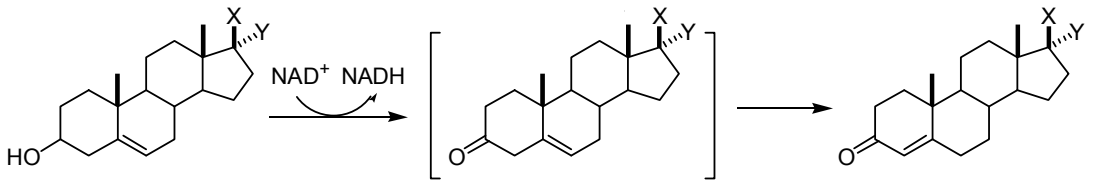
Table 2-2. Tryptic Peptides from $rH_6$3βHSD identified by MALDI-TOF mass spectrometry.

Residues	Sequences[a]
1-50	MGSSHHHHHHSSGLVPRGSHMLRRMGDASLTTELGR**VLVTGGAGFVGANL**
51-100	**VTTLLDR**GHWVRSFDRAPSLLPAHPQLEVLQGDITDADVCAAAVDGIDTI
101-150	FHTAAIIELMGGASVTDEYRQR**SFAVNVGGTENLLHAGQRAGVQR**FVYTS
151-200	SNSVVMGGQNIAGGDETLPYTDRFNDLYTETKVVAERFVLAQNGVDGMLT
201-250	CAIRPSGIWG NGDQTMFRKLFESVLKGHVKVLVGRKSARLDNSYVHNLIH
251-300	GFILAAAHLVPDGTAPGQAY FINDAEPINMFEFARPVLEACGQRWPKMRI
301-350	SGPAVR**WVMTGWQR**LHFR**FGFPAPLLEPLAVERLYLDNYFSIAK**ARRDLG
351-392	YEPLFTTQQA LTECLPYYVSLFEQMKNEARAEKTAATVKPKL

[a] Identified sequences are shown in bold-face, underlined, amino acids are given in single-letter code. [reprinted with permission from (1); copyright 2007, American Chemical Society].

Reactions catalyzed by the recombinant enzyme. $rH_6$3βHSD was assayed with three sterols: cholesterol, pregnenolone, and dehydroepiandrosterone, two flavonols: quercetin and dihydroquercetin, and nicotinamide cofactors. The cofactor or the sterols were omitted from the assay mixtures as negative controls. Ketone product was formed only in reaction mixtures containing NAD^+ and 3β-hydroxysterol. $NADP^+$ did not function as a cofactor with any of the three sterols at concentrations up to 10 mM. The cofactor preference is analogous to that reported for the *Nocardia* cholesterol dehydrogenase (*30*) and consistent with the conservation of Asp^{45} in the short chain alcohol dehydrogenase family. $rH_6$3βHSD did not catalyze oxidation of quercetin with NAD^+ or reduction of dihydroquercetin with NADH. The sterol reaction mixtures were analyzed by TLC and products compared to authentic standards. The enzyme catalyzed the

oxidation and isomerization of cholesterol, pregnenolone, and dehydroepiandrosterone into cholest-4-en-3-one, progesterone, and androsterone, respectively (Scheme 2-1). In addition, the cholesterol reaction course was followed by reversed-phase C-18 chromatography, and the identities of the intermediate and product, cholest-5-en-3-one and cholest-4-en-3-one, confirmed by co-injection with standards. The *Rv1106c* open-reading frame clearly encodes for a 3β-hydroxy sterol dehydrogenase, not a dihydroflavonol reductase.



cholesterol, cholestenone: X=$CH_3CH(CH_2)_3CH(CH_3)_2$, Y=H
pregnenolone. progesterone: X=$COCH_3$, Y=H
dehydroepiandrosterone, androsterone: X,Y=O

Scheme 2-1. Reaction catalyzed by *M. tb* 3β-hydroxysteroid dehydrogenase (*Rv1106c).* [reprinted with permission from (1); copyright 2007, American Chemical Society].

The $rH_6$3βHSD enzyme activity with cholesterol was measured as a function of pH and the rate plateaued at pH 8.5. Next the enzyme activity was measured under four different detergent conditions: 0.05% triton X-100, 2% triton X-100, 1% nonyl glucoside, and 1% sodium cholate. There was no activity in nonyl glucoside, and very little activity in sodium cholate. The highest activity was obtained with 2% triton X-100. However, the enzyme activity was not saturated with cholesterol in any of the detergent systems. The highest cholesterol concentration that could reliably be attained was 1 mM cholesterol in 2% triton X-100. At this cholesterol concentration, the specific activity of the $rH_6$3βHSD was 2.90 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ in 100 mM triethanolamine hydrochloride, pH 8.5.

Dehydroepiandrosterone and pregnenolone have sufficient aqueous solubility that the use of detergent micelles is not required. Both substrates yielded normal saturation kinetics with the *M. tb* dehydrogenase. The lack of saturation with cholesterol may be due to cholesterol being a poor substrate for the enzyme. Alternatively, it may be due to a low affinity of the enzyme for the detergent micelle surface. After optimization of pH and cation conditions (*vide infra*), $rH_6$3βHSD had the highest specific activity with dehydroepiandrosterone as the substrate; the activity was 2.5-3 fold higher with dehydroepiandrosterone than with cholesterol in 0.05% triton X-100 or pregnenolone. The same relative activities were obtained when each of the three sterols was assayed in the presence of 0.05% triton X-100 in the optimized buffer conditions of 100 mM TAPS hydrochloride, 150 mM NaCl and 30 mM $MgCl_2$ pH 8.5 and 2.8 mM NAD^+ (*vide infra)*. The specific activities measured were 0.15, 0.36, and 0.28 μM min^{-1} mg^{-1} for cholesterol, dehydroepiandrosterone, and pregnenolone, respectively. Dehydroepiandrosterone was used as the substrate in subsequent studies because its solubility and kinetic behavior made it the most amenable to the detailed analysis performed.

Both the recombinant *Rv1106c* with ($rH_6$3βHSD) and without (r3βHSD) an N-terminal histidine tag were assayed with a fixed concentration of dehydroepiandrosterone (300 μM) and NAD^+ was varied from 1-28 mM in 100 mM triethanolamine hydrochloride, pH 8.5, 30 °C. The apparent K_m's for NAD^+ were 2.4 ± 0.7 mM and 2.2 ± 0.3 mM, respectively, demonstrating that the N-terminal histidine tag did not affect substrate binding. Because the r3βHSD protein was expressed at low levels and could not be purified to homogeneity, its k_{cat} could not be accurately determined. All subsequent experiments were performed with N-terminally His-tagged enzyme $rH_6$3βHSD because it was expressed at higher levels and was easier to purify.

The substrate specificities reported for the *Nocardia* cholesterol dehydrogenase were similar, but not identical to those observed for $rH_6$3βHSD (*30*). The *Nocardia* enzyme utilizes both cholesterol and pregnenolone, whereas no activity with dehydroepiandrosterone was observed. However, the assay conditions used for the latter sterols were not reported. Therefore, it is unclear if the difference in specificity is due to buffer conditions or true differences between the enzymes. Kishi et al. reported a K_m of 2.5 mM for cholesterol in 1.87% Triton X-100 for the *Nocardia* cholesterol dehydrogenase (*30*). However, the highest cholesterol concentration used was 2.9 mM, suggesting that as with the *M. tb* enzyme studied in this work, saturation of the *Nocardia* enzyme with cholesterol was not possible.

pH dependence. Initial velocity kinetic patterns and apparent kinetic constants K_m and k_{cat} with dehydroepiandrosterone as the substrate were measured at a fixed concentration of NAD^+ (2.8 mM) in 100 mM TAPS hydrochloride with 150 mM NaCl and 30 mM $MgCl_2$ at pH 9.5, 9.0, 8.5, 8.0, 7.5, 7.0 and 6.5. No catalytic activity was detected below pH 7.5. The apparent pK_a for k_{cat} is 7.9 ± 0.1 and the apparent pK_a for k_{cat}/K_m is 8.1 ± 0.1. The pH optimum of the enzyme is pH 8.5-9.5 (Figure 2-2). This pH optimum range is comparable to that reported for the *Nocardia* cholesterol dehydrogenase (*30*). Further experiments were conducted at pH 8.5 to optimize activity while minimizing decomposition of the NAD^+ cofactor during the assay.

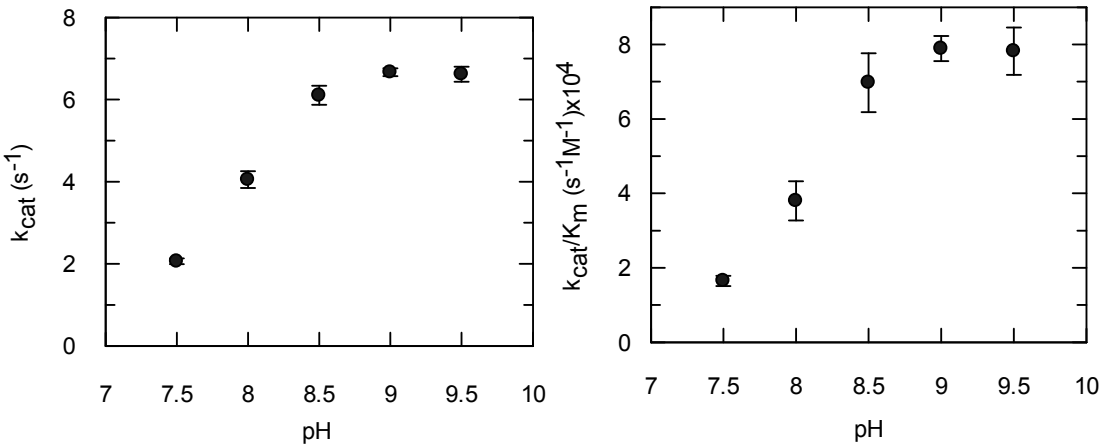


Figure 2-2. pH dependence of the reaction catalyzed by $rH_6$3βHSD with DHEA as the varied substrate. Conditions: 2.8 mM NAD^+, 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5, 30 °C. A, pH dependence of k_{cat}. B, pH dependence of k_{cat}/K_m. The curves are fits to eq. (7). The data shown are the average of three independent experiments, and the errors are the standard deviation of measurement. [reprinted with permission from (1); copyright 2007, American Chemical Society].

Cation and ionic strength dependence. $rH_6$3βHSD was assayed with 10 mM and 1 mM $CaCl_2$, $MgCl_2$, 1 mM $FeCl_2$, $CuCl_2$, $ZnCl_2$, or $AgNO_3$, $CuCl_2$ or 50 mM EDTA in the presence of 150 μM dehydroepiandrosterone, and 3.5 mM NAD^+ in 100 mM triethanolamine hydrochloride, pH 8.5 (Figure 2-3A). EDTA, Fe^{2+}, Zn^{2+}, Ag^+ and Cu^{2+} inhibited enzymatic activity. Mg^{2+} and Ca^{2+} were the best activators of enzymatic activity. Then, $rH_6$3βHSD was assayed as a function of NaCl concentration in the presence of 150 μM dehydroepiandrosterone and 2.8 mM NAD^+ in 100 mM TAPS hydrochloride, pH 8.5 (Figure 2-3B). The activity increased with increasing NaCl and plateaued at about 300 mM. A concentration of 150 mM NaCl was used in subsequent assays as this represented a physiological ionic strength. Next, $rH_6$3βHSD was assayed with 10 mM and 1 mM $CaCl_2$, $MgCl_2$, 1 mM $FeCl_2$, $CuCl_2$, $ZnCl_2$, or $AgNO_3$, $CuCl_2$ or 50 mM EDTA in the presence of 150 μM dehydroepiandrosterone, 150 mM NaCl, and 2.8 mM NAD^+ in 100 mM TAPS hydrochloride, pH 8.5 (Figure 2-3C). The highest activities were observed with Ca^{2+}, Mg^{2+} and K^+. Different concentrations of Mg^{2+} were assayed in the presence

of 150 mM NaCl and and the activity plateaued between 20 mM and 40 mM (Figure 2-3C). Subsequent assays were performed with 150 mM NaCl and 30 mM $MgCl_2$.

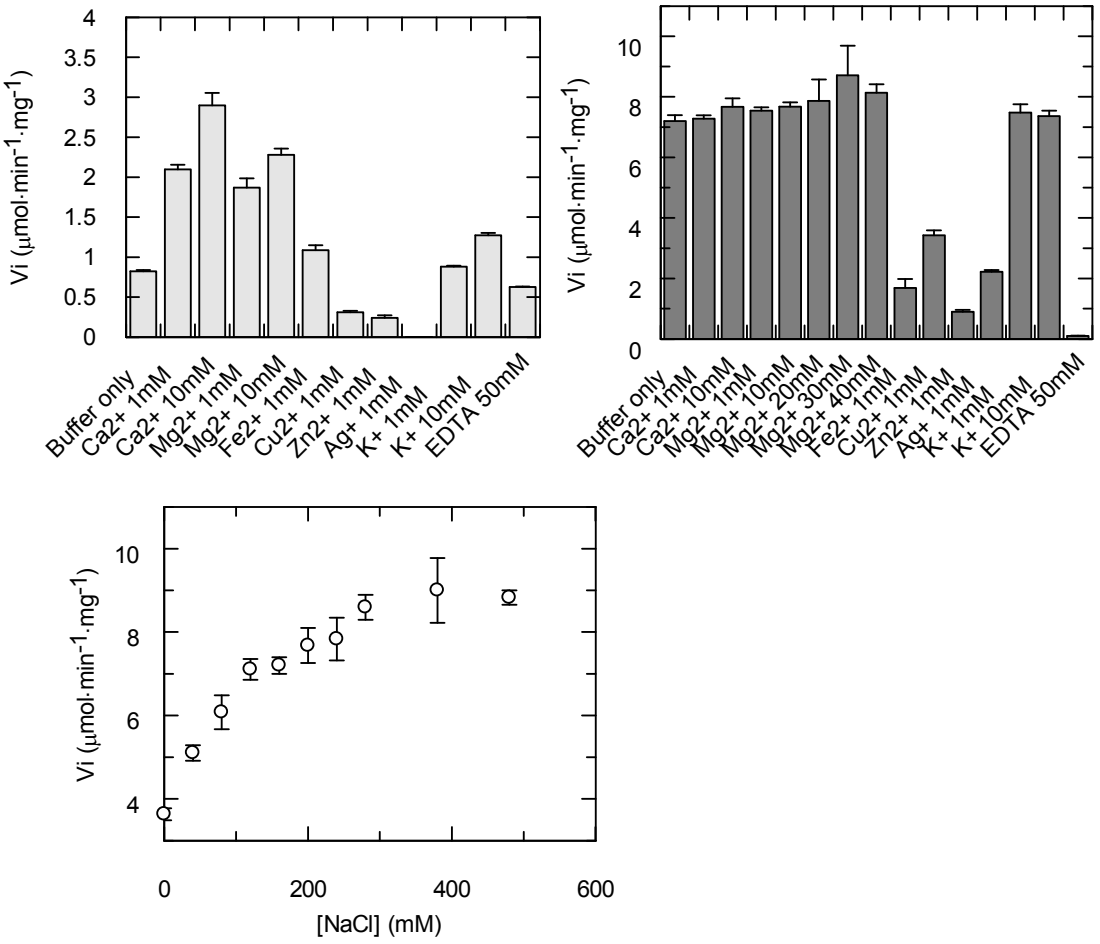


Figure 2-3. Cation dependence of $rH_6$3βHSD activity. A, Specific activity in the presence of assorted cations or EDTA. Conditions: 150 μM DHEA, 3.5 mM NAD^+, 100 mM triethanolamine hydrochloride buffer, pH 8.5, 30 °C. B, Specific activity as a function of NaCl concentration. Conditions: 150 μM DHEA, 2.8 mM NAD^+, 100 mM TAPS hydrochloride buffer, pH 8.5, 150 mM NaCl, 30 °C. C, Specific activity in the presence of 150 mM NaCl and assorted cations or EDTA. Conditions: 150 μM DHEA, 2.8 mM NAD^+, 100 mM TAPS hydrochloride buffer, pH 8.5, 150 mM NaCl, 30 °C. The data shown are the average of three independent experiments, and the errors are the standard deviation of measurement. [reprinted with permission from (1); copyright 2007, American Chemical Society].

Steady-state kinetic assays. Initial velocity data with $rH_6$3βHSD and varied substrate concentrations produced an intersecting line pattern (Figure 2-4) consistent with a sequential binding mechanism as is expected for an alcohol dehydrogenase

reaction. Concentrations of NAD^+ higher than 5.6 mM inhibit the dehydrogenase reaction (Figure 2-5). The substrate inhibition by NAD^+ could be overcome by increasing the concentration of dehydroepiandrosterone. This competitive inhibition suggests that NAD^+ either binds to the E-NAD^+ complex to form a dead-end E-$(NAD^+)_2$ complex or that the E-NAD^+ complex is itself a dead-end complex. Which species is formed depends on the order of substrate binding in the enzyme-catalyzed reaction. Further analysis with additional inhibitors is required to determine the reaction order (*vide infra*).

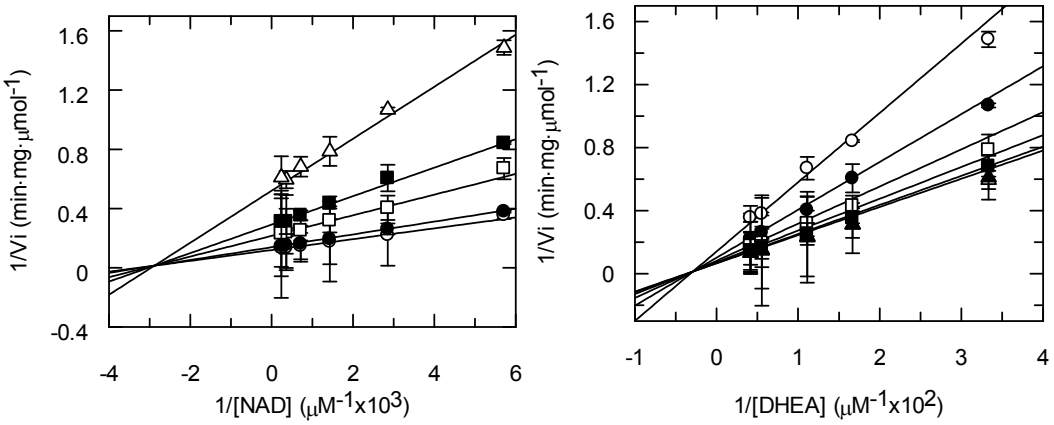


Figure 2-4. Two-substrate steady state kinetics of $rH_6$3βHSD. Double-reciprocal plots of initial velocity against NAD^+ or DHEA at varied DHEA or NAD^+ were fitted to equation (1). Conditions: 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5, 30 °C. The data shown are the average of three independent experiments, and the errors are the standard error of measurement. A, Double-reciprocal plot, 1/V versus 1/[NAD^+], at varied DHEA concentrations: ∆, 30 μM; ■, 60 μM; □, 90 μM; ●, 180 μM; and ○, 240 μM. B, Double-reciprocal plot, 1/V versus 1/[DHEA], at varied NAD^+ concentrations: ○, 175 μM; ●, 350 μM; □, 700 μM; ■, 1400 μM; ∆, 2800 μM; and ▲, 4200 μM. [reprinted with permission from (1); copyright 2007, American Chemical Society].

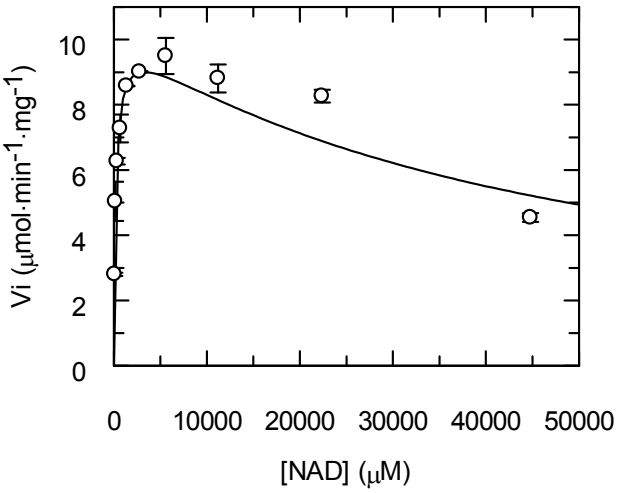


Figure 2-5. Steady state kinetics of $rH_6$3βHSD. A plot of initial velocity against NAD^+ at fixed DHEA was fitted to equation (5). Conditions: 120 μM DHEA, 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5, 30 °C. The data shown are the average of three independent experiments, and the errors are the standard deviation of measurement. [reprinted with permission from (1); copyright 2007, American Chemical Society].

Because of substrate inhibition, the highest concentration of NAD^+ used for fits of initial velocity data to Equation (1) was 4.2 mM. The ternary complex steady-state kinetic parameters were derived by globally fitting the initial velocity data to Equation (1) for an ordered reaction (Table 2-3). Inhibitors of the reaction catalyzed by $rH_6$3βHSD were required in order to determine the order of substrate binding. A steroid-derived inhibitor and a cofactor-derived inhibitor were sought.

Table 2-3. Michaelis-Menten Constants for $rH_6$3βHSD.

Substrate A	Substrate B	K_{ia} (μM)[a]	K_{ma} (μM)[a]	K_{mb} (μM)[a]	k_{cat}(s^{-1})[a]	k_{cat}/K_{mb} ($\mu M^{-1}min^{-1}$)
NAD^+	DHEA	347 ± 28	270 ± 37	221 ± 22	11.6 ± 0.73	3.2 ± 0.4
NAD^+	pregnenolone	213 ± 162	172 ± 52	23 ± 5	1.48 ± 0.09	3.9 ± 0.9

[a]A compulsory order ternary-complex mechanism as described in equation (1) was fit to the initial velocity data to yield K_{ia}, K_{mB}, and K_{mA}. Conditions: 100 mM TAPS, 150 mM NaCl, 30 mM $MgCl_2$ buffer, pH 8.5, 30 °C. [reprinted with permission from (1); copyright 2007, American Chemical Society].

Trilostane {(2α,4α,5α,17β)-4,5-epoxy-17-hydroxy-3-oxoandrostane-2-carbonitrile} is a known competitive inhibitor (*versus* pregnenolone) of mammalian 3β-hydroxysteroid dehydrogenases (*31*). It was expected that trilostane would be an inhibitor of the *M. tb* 3β-hydroxysteroid dehydrogenase as well because the reactions catalyzed by both enzymes, and thus, the substrates, intermediates, and products, are the same. Indeed, trilostane is a micromolar inhibitor of $rH_6$3βHSD (Figure 2-6). Moreover, the cofactor product of oxidation, NADH, was also found to be an inhibitor of the $rH_6$3βHSD-catalyzed reaction. The order of the reaction catalyzed by $rH_6$3βHSD was determined by analyzing patterns of inhibition by trilostane and NADH (Table 2-4, Figures 2-6 and 2-7). Competitive inhibition by trilostane with respect to dehydroepiandrosterone and competitive inhibition by NADH with respect to NAD^+ suggested that the reaction has a compulsory order. If the order of binding were random, mixed inhibition would have been observed. Uncompetitive inhibition by trilostane with respect to NAD^+ and mixed inhibition by NADH with respect to DHEA indicate that the NAD^+ binds to the enzyme first followed by DHEA to form a ternary complex. This order of reaction is observed for a large number of NAD(P)-dependent dehydrogenases. This information was used to fit an ordered ternary mechanism to the kinetic data acquired for both dehydroepiandrosterone and pregnenolone as substrates. Although k_{cat} is larger for dehydroepiandrosterone, the enzyme has a larger K_m for this substrate compared to pregnenolone. Thus, the second-order or specificity rate constants (k_{cat}/K_m) are approximately the same for both steroids. A similar lack of specificity has been observed for the human 3β-hydroxysteroid dehydrogenases (*32*). The human enzymes catalyze the dehydrogenase reaction at second-order rates similar to those catalyzed by $rH_6$3βHSD.

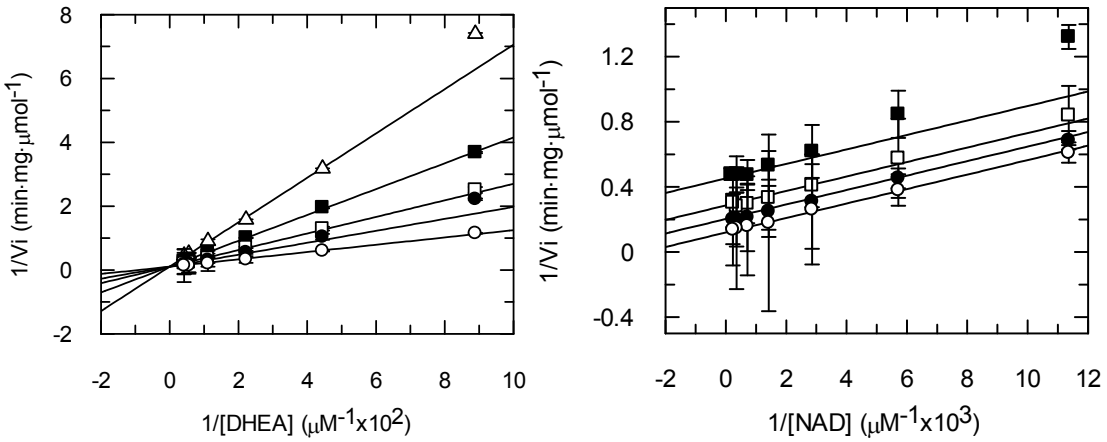


Figure 2-6. Trilostane inhibition kinetics of $rH_6$3βHSD. Double-reciprocal plot of initial velocity against DHEA or NAD^+ at varied trilostane was fitted to equation (2 or 3). Trilostane concentrations used were: Δ, 1000 μM; ■, 500μM; □, 250μM; ●, 125μM; and ○, 0 μM. Conditions: 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5, 30 °C. The data shown are the average of three independent experiments, and the errors are the standard error of measurement. A, Double-reciprocal plot against DHEA at fixed NAD^+ (350 μM). B, Double-reciprocal plot against NAD^+ at fixed DHEA (120 μM). [reprinted with permission from (1); copyright 2007, American Chemical Society].

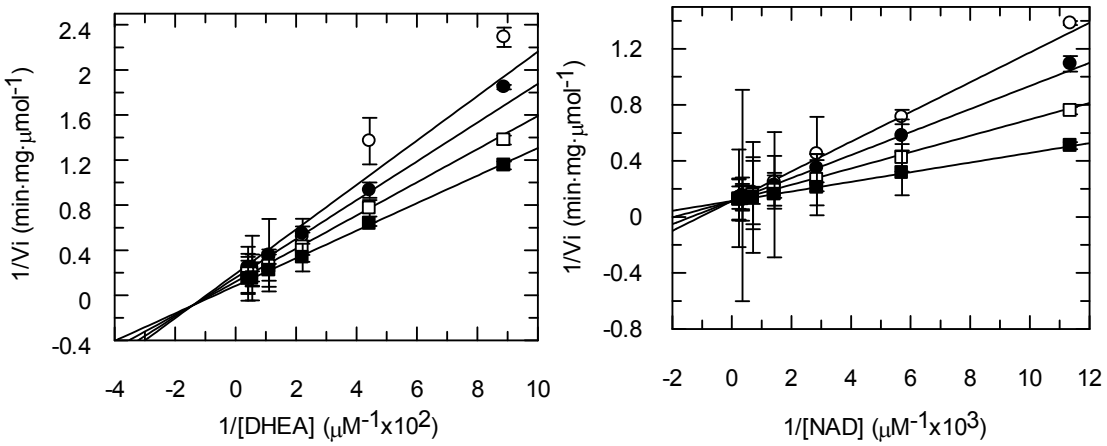


Figure 2-7. NADH inhibition kinetics of $rH_6$3βHSD. Double-reciprocal plot of initial velocity against DHEA or NAD^+ at varied NADH was fitted to equation (4 or 2). NADH concentrations used were: ○, 150 μM; ●, 100μM; □, 50μM; and, ■, 0 μM. Conditions: 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5, 30 °C. The data shown are the average of three independent experiments, and the errors are the standard error of measurement. A, Double-reciprocal plot against DHEA at fixed NAD^+ (350 μM). B, Double-reciprocal plot against NAD^+ at fixed DHEA (90 μM). [reprinted with permission from (1); copyright 2007, American Chemical Society].

Table 2-4. Inhibition of $rH_6$3βHSD.

Inhibitor	*Versus* DHEA			*Versus* NAD^+		
	Pattern type	K_{ic} (μM)[a]	K_{iu} (μM)[a]	Pattern type	K_{ic} (μM)[a]	K_{iu} (μM)[a]
trilostane	C[b]	197 ± 8[c]	n.a.[d]	UC	n.a.[d]	180 ± 11[e]
NADH	mixed	245 ± 68	116 ± 22	C	72 ± 3	n.a.[d,f]

[a]A compulsory order ternary-complex mechanism was fit to equations 2-4 with the initial velocity data and the best fit was used to yield K_{ic}, or K_{iu}. Conditions: 100 mM TAPS hydrochloride buffer, pH 8.5, 150 mM NaCl, 30 mM $MgCl_2$, 30 °C. [b]C: competitive inhibition; UC: uncompetitive inhibition; mixed: mixed inhibition. [c]NAD^+ was fixed at 350 μM. [d]n.a., not applicable. [e]DHEA was fixed at 120 μM. [f]DHEA was fixed at 90 μM. [reprinted with permission from (1); copyright 2007, American Chemical Society].

Furthermore, the competitive substrate inhibition by NAD^+ that is observed must be due to the formation of an E-$(NAD^+)_2$ dead-end or non-productive complex. The structural explanation for how this complex may be formed awaits three-dimensional structural data that is unavailable at present. The initial velocity data *versus* NAD^+ at a single fixed dehydroepiandrosterone concentration of 120 μM (shown in Figure 2-5) were fit to Equation (5), and K_{iNAD+} was determined to be 46.3 ± 7.7 mM. To confirm this value, the competitive substrate inhibition constant was derived by determining the apparent K_m and V_m values from all of the initial velocity *versus* dehydroepiandrosterone plots using Equation (1) and replotting K_m/V_m *versus* NAD^+ (Figure 2-8). The K_m/V_m data for NAD^+ concentrations above 5.6 mM were fit linearly and K_{iNAD+} was determined to be 40.5 mM (Figure 2-8). Both analysis methods yielded the same inhibitory dissociation constant confirming that it is not dependent on dehydroepiandrosterone concentration.

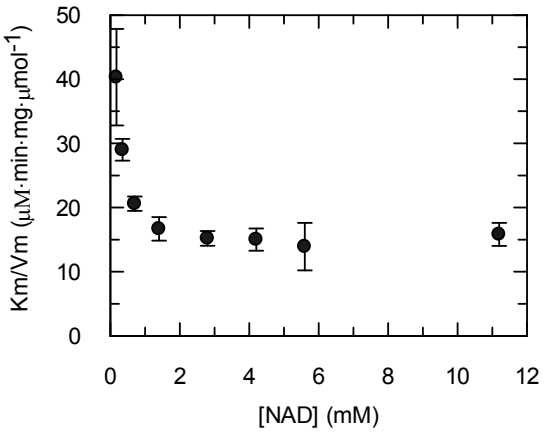


Figure 2-8. NAD^+ inhibition kinetics of $rH_6$3βHSD. Secondary plot of K_m/V_m against NAD^+. Conditions: 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5, 30 °C. [reprinted with permission from (1); copyright 2007, American Chemical Society].

In vivo analysis of Rv1106c expression. The substrate specificity and inhibitor analyses presented in this work suggest that *Rv1106c* encodes a 3β-hydroxysteroid dehydrogenase. To determine whether *Rv1106c* is the gene responsible for 3β-hydroxysterol oxidation *in vivo*, the expression of *Rv1106c* in wild-type *M. tb* (CDC1551) and in a transposon mutant in which the *MT1137* gene (corresponding to *Rv1106c* in the H37Rv strain) had been disrupted was investigated.

Wild-type *M. tb* and *MT1137* mutant CDC1551 were grown in Middlebrook 7H9 medium that was supplemented with 10% albumin-dextrose-sodium chloride complex, 0.2% glycerol and 0.05% Tween-80 to mid-log phase. Then, cholesterol (1 mg/mL) solubilized in Tween-80 was added to the cultures and the bacteria were cultured for an additional 24 h. The cell culture supernatants and soluble fraction of the cell lysate were assayed for their ability to oxidize cholesterol using an HPLC assay in which cholesterol and cholest-4-en-3-one were detected. The HPLC assay used was not specific for dehydrogenase activity (e.g., versus oxidase activities), it reports on all cholesterol oxidizing activities that may be present. Cholesterol oxidation activity (~0.07 units/liter,

two independent experiments) was observed in the soluble fraction of the wild-type cell lysate. No cholesterol oxidation activity was observed in the *MT1137* mutant cell lysates. Under the conditions of the HPLC assay used, the limit of detection is 0.0008 units/liter. Thus, disruption of the *MT1137* gene reduces the cholesterol oxidation activity of *M. tb* at least 90-fold. In addition, no cholesterol oxidation activity was observed in either the wild-type or mutant culture filtrates.

The implications of this experiment are several-fold. First, upon disruption of a single gene, all detectable cholesterol oxidation activity is abrogated. This result implies that there is a single sterol-oxidizing enzyme, and that this enzyme is the *Rv1106c*-encoded 3β-hydroxysteroid dehydrogenase. Second, cholesterol oxidation activity was observed only in cell lysates; no activity was observed in culture filtrates. This result suggests that *M. tb* does not secrete the 3β-hydroxysteroid dehydrogenase encoded by *Rv1106c.* This observation is consistent with a proteomic analysis that identified the *M. tb Rv1106c* gene product in the cytosol (*33-34*). Third, disruption of the enzymatic activity with a single knockout bodes well for the discovery of small molecule inhibitors that can completely inhibit *M. tb* cholesterol oxidation *in vivo*. To further validate the hypothesis, host-infection virulence studies with wild type and mutant strains will be discussed in Chapter 4.

Mutagenesis studies. As shown in Figure 2-9, the sequence alignment of *M. tb* 3βHSD and the related proteins among the SDR superfamily revealed the presence of conserved cofactor binding residues (Gly21, Gly27, and Asp45) and active-site residues (Asn107, Ser131, Tyr158 and Lys162) in *M. tb* 3βHSD. Point mutations of 3β-hydroxysteroid dehydrogenase, N107Q, S131A, Y158F, and K162I, were constructed. The corresponding proteins were heterologously expressed in *E. coli* and purified as previously described for wild-type $rH_6$3βHSD. The isolated proteins were determined to have MWs of 43 kD by SDS-PAGE analysis and the protein identities were confirmed by

MALDI-TOF mass analysis of tryptic peptide mixtures generated by in-gel digestion. No detectable 3β-hydroxysteroid dehydrogenase activity was observed for the S131A, Y158F, and K162I mutants, with substrate concentrations of dehydroepiandrosterone at 150 μM and NAD^+ at 1.4 mM, and enzyme concentrations up to 1 μM. Numerous studies reveal that the Tyr functions as the catalytic base, Ser stabilizes the substrate and Lys together with a positively charged nicotinamide ribose lowers the pK_a of the hydroxyl group in Tyr (*35*-*38*). The dramatic kinetic difference between the wild type and the mutants of S131A, Y158F and K162I provide evidence in support of the Tyr^{158}-Thr-Glu-Thr-Lys^{162} motif (*39*) being the catalytic center in *M. tb* 3βHSD (Figure 2-10). Mutation of those residues also resulted in inactivation of other extended SDR members, such as *Drosophila* alcohol dehydrogenase (*40*) and human 3β-hydroxysteroid dehydrogenase (*41*). The crystal structure of *Drosophila* ADH demonstrated the interaction of the conserved Asn111 binding to the active site Lys via a water molecule (*42*). The apparent k_{cat} of N107Q mutant was over 100-fold lower compared to that of the wild-type 3βHSD (Table 2-5) and the K_m was slightly decreased. One possible explanation is that the substrate binds to the enzyme in which asparagine is mutated to glutamine. However, the mutation hinders further binding of the cofactor to the active site and therefore compromises the reaction. Overall, the mutagenesis studies fit the active site characteristics of most SDR proteins: a catalytic tetrad of Asn-Ser-Tyr-Lys residues. However, further mutagenesis and crystallography studies are required to validate the active site architecture and reaction mechanism in *M. tb* 3βHSD.

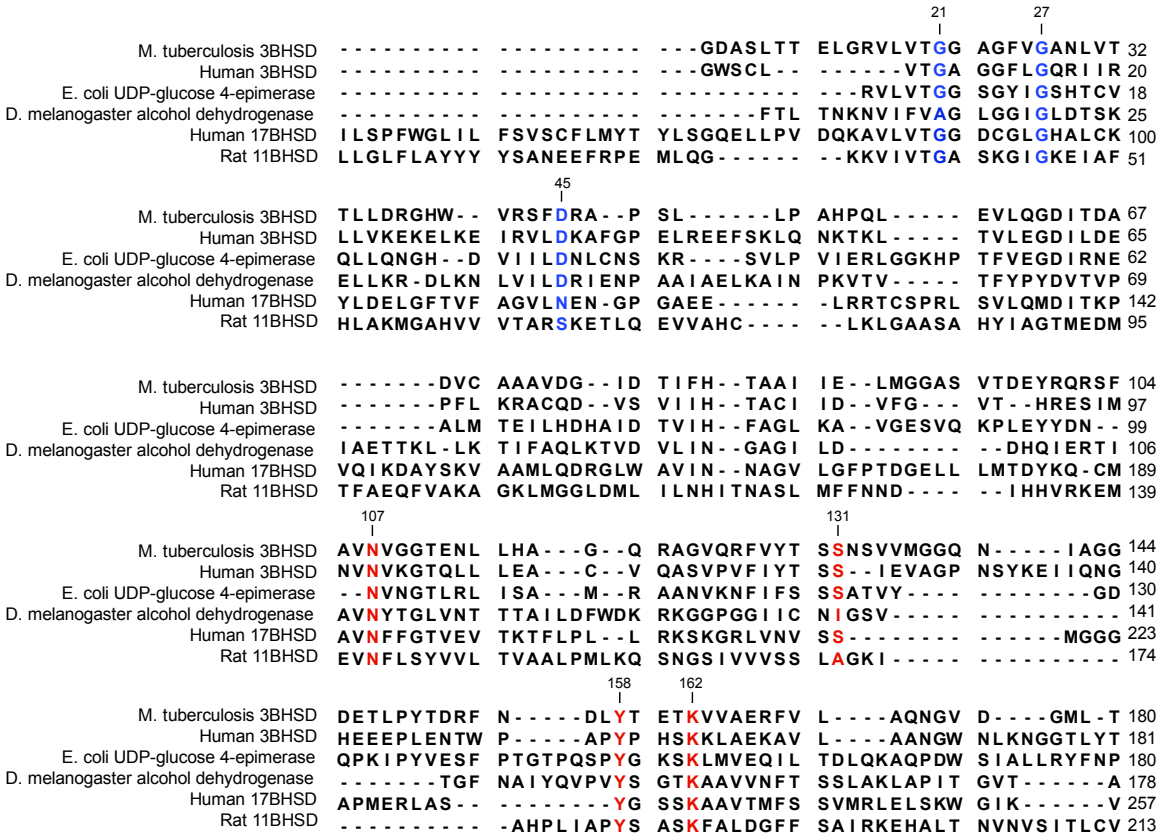


Figure 2-9. Sequence alignment (partial) for 3β-hydroxysteroid dehydrogenase encoded by *Rv1106c* and related genes. Putative cofactor binding residues are in blue and putative active-site residues are in red. The alignment was generated using CLC Free Workbench 3.

Table 2-5. Substrate kinetics for the 3βHSD mutants.

Enzyme	K_M^{app}(μM)	k_{cat}^{app} (s^{-1})	k_{cat}^{app} / K_M^{app} ($s^{-1}\cdot\mu M^{-1}$)
Wild-type	218	9.57	0.044
N107Q	94.5	0.07	0.00075
S131A	N.D.	No activity	N.D.
Y158F	N.D.	No activity	N.D.
K162I	N.D.	No activity	N.D.

Kinetic constants were determined with a fixed NAD^+ concentration at 1.4 mM. Conditions: 100 mM TAPS hydrochloride buffer, pH 8.5, 150 mM NaCl, 30 mM $MgCl_2$, 30 °C.

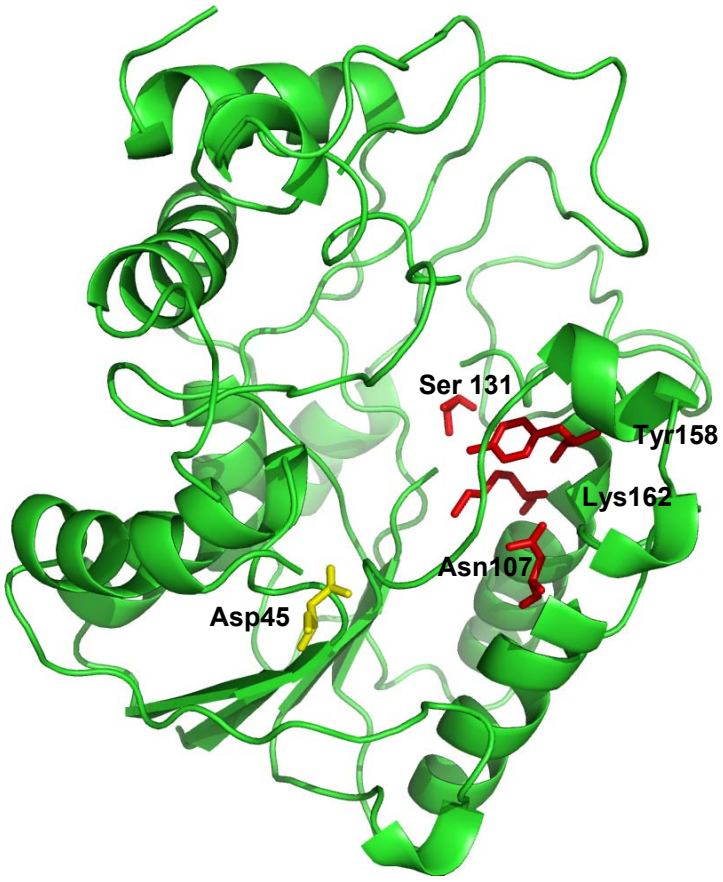


Figure 2-10. Ribbon diagram of 3βHSD based on homology modeling using UDP-galactose-4-epimerase (PDB code 1A9Z) as a template.

Summary. Mycobacteria and related actinomycetes have long been reported to oxidize environmental cholesterol (*43-44*). However, the actual identity and genotype of the strains isolated and studied has not always been clear (*45*). With the recent complete sequencing of several bacterial genomes including that of *M. tb* (*46-47*), it is now possible to connect bacterial phenotypes to specific genes. In this work, genome mining was used to identify a potential 3β-hydroxysterol oxidizing enzyme from *M. tb*, the product of gene *Rv1106c*.

Elucidation of the kinetic profile for the heterologously expressed *M. tb Rv1106c* gene product clearly establishes that the enzyme is a 3β-hydroxysteroid dehydrogenase that oxidizes the 3-hydroxyl and isomerizes the α,β-unsaturated ketone into the

conjugated α,β-unsaturated ketone of at least three sterol substrates, cholesterol, dehydroepiandrosterone, and pregnenolone. Under the conditions optimized in this work, the enzyme is equally specific for dehydroepiandrosterone and pregnenolone, and three-fold less specific for cholesterol. Although the maximal catalytic activity is 10-fold lower with pregnenolone as a substrate, the apparent binding constant (K_m) for pregnenolone is 10-fold better (Table 2-3). Thus, the *in vivo* substrate concentrations, and in the case of cholesterol, membrane composition will dictate the substrate selection. At present, these concentrations and membrane compositions are unknown. Like most of the members in SDR superfamily, the conserved active site pocket containing Asn-Ser-Tyr-Lys motif is likely involved in the *M. tb* 3βHSD catalytic reactions.

The intracellular expression of the *M. tb Rv1106c* gene product is more consistent with the use of 3β-hydroxy sterols for steroid hormone biosynthesis, for example, by mycobacterial conversion of cholesterol into glucocorticoids as seen in the case of vaccinia virus (*17*) or androgens, rather than a role in energy metabolism. All characterized examples of actinomycetes, e.g., *Rhodococcus* (*48-49*), *Streptomyces (50)*, and *Pseudomonas* (*51*), and some proteobacteria, e.g., *Sterolibacterium* (*52-53*), catabolize 3β-hydroxysterols for energy use and secrete the sterol oxidizing enzyme. Lastly, in contrast to the *M. tb Rv1106c* gene product, 3β-hydroxy sterol oxidases known to be involved in primary metabolism are more specific for cholesterol than dehydroepiandrosterone or pregnenolone (*54-56*). The intracellular expression of the enzyme suggests that 3β-hydroxysterols must be taken into the mycobacterium from its environment, e.g., the host cell macrophage. An alternative possibility is that the enzymatic substrate is synthesized by the mycobacterium. However, no evidence for the mycobacterial biosynthesis of cholesterol has yet been obtained (*57-58*). Future experiments will explore the possible physiological and pathological roles of this enzyme in steroid biosynthesis and degradation.

ACKNOWLEDGMENT

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Chapter 3

3β-hydroxysteroid dehydrogenase inactivation *in vitro* and *in vivo*

I. Introduction

The *in vivo* importance of *hsd* can be determined by both the activities of inhibitors and gene disruption. We looked to previously developed inhibitors and found that inhibitors of 5α-reductase are candidate inhibitors of *M. tb* Hsd. At the same time, we investigated the phenotype of the *hsd* mutant *in vivo*.

The enzyme 5α-reductase catalyzes the transfer of a hydride from NADPH to the $\Delta^{4,5}$ double bond of various steroids, e.g., the conversion of testosterone to a more potent intracellular hormone, 5α-dihydrotestostrone (DHT). Inhibition of this enzyme provides a potent therapy for the androgen-related disorders (*1*). 4-azasteroids were initially synthesized as potent reversible inhibitors of hepatic and prostatic steroid 5α-reductase (*2*). Finasteride, one of most extensively studied 4-azasteroids, is a competitive inhibitor of type 2 5α-reductase and is effective in the treatment of benign prostatic hypertrophy and male androgenic alopecia (*3-4*). However, one obstacle of this inhibitor is the relative slow rate of inhibition of type 1 5α-reductase (*5*), which leads to some pharmacodynamic problems (*6-7*). In a search for potent dual inhibitors of 5α-reductase, the 6-azasteroids series was developed by GlaxoSmithKline and their *in vivo* and *in vitro* pharmaceutical studies were fully profiled (*8-11*). A structure-activity relationship was performed in order to optimize potency versus both isozymes (*8*). By further modifying C17 substituents of 4-azasteroids, dutasteride was designed as a dual inhibitor to type 1 and type 2 5α-reductase (*12*). Oral dutasteride is approved for the treatment of moderate to severe symptomatic benign prostatic hyperplasia in men (*13*).

However, some 4-azasteroids show non-specific inhibition of 5α-reductase, e.g., N,N-diethyl-4-methyl-3-oxo-4-aza-5α-androstane-17β-carboxaminde blocks the conversion of pregnenolone to progesterone (*14*) and was used as an inhibitor of mammalian Hsd for the enzymatic mechanism studies (*15*). One structural explanation

for the inhibition is that those inhibitors may mimic the Δ^3-enolate intermediate generated during catalysis (*16*). One direction of the structural modification of 4-azasteroids and 6-azasteroids as drugs for benign prostatic hyperplasia was to design inhibitors with optimized potency versus both isozymes of 5α-reductase and selectivity versus mammalian Hsd (*17*). In contrast, we performed inhibition screening with those 4- or 6-azasteroids against *M. tb* Hsd in order to find better inhibitors of Hsd and help understand the inhibition mechanism as well as the structure of Hsd.

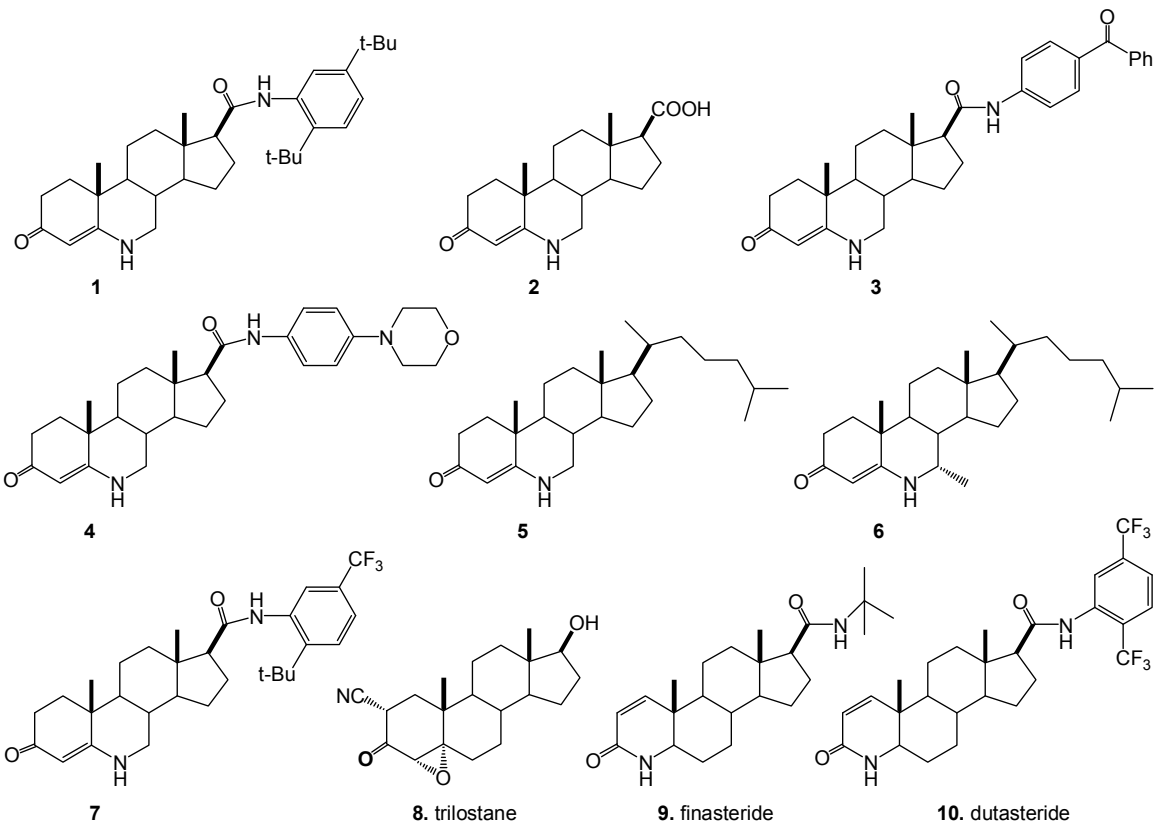
In an attempt to explore the crystal structure of the enzyme, we performed high-throughput screening of *M. tb* Hsd to identify the initial crystallization conditions.

II. Materials and methods

Materials. NAD^+, NADH, dehydroepiandrosterone, androsterone, and finasteride were purchased from Sigma-Aldrich (St. Louis, MO). Trilostane and dutasteride were from AK Scientific, Inc (Mountain View, CA). 6-azasteroids were provided by GlaxoSmithKline for inhibition tests. Buffer A: 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5. Experiments with guinea pig infection model were performed by TARGET program. All other experiments with *M. tb* and animals were performed at PHRI, UMDNJ by Drs. Eugenie Dubnau and Irina Kolesnikova.

Determination of inhibitor K_i values. The 3β-hydroxysteroid dehydrogenase (*M. tb* Hsd) was purified and the enzyme activities were measured as described in Chapter 2. A dehydroepiandrosterone stock solution was prepared in ethanol (3 mM). Inhibitors were initially dissolved in DMSO and diluted in buffer A. The final volume of alcohol was 5% (v/v) and the final volume of DMSO was 2% (v/v) in all assays. Enzyme activities were measured in the presence of constant substrate concentrations

(dehydroepiandrosterone at 150 μM and NAD^+ at 3.5 mM) and several inhibitor concentrations for inhibitors **1**-**10** (Scheme 3-1). Initial velocities were fit to Eq. 1 using Grafit (version 4.0.10) and K_i*s* were calculated using Eq. 2.



Scheme 3-1. Structures of inhibitors.

Mechanism of inhibition against M. tb Hsd. Initial velocities were measured for varying finasteride concentrations (0 - 250 μM) at different fixed concentrations of DHEA (88 - 5600 μM) with NAD^+ at a concentration of 1 mM or varying GI190639x concentrations (0 – 38.3 μM) at different fixed concentrations of DHEA (15-150 μM) with NAD^+ at a concentration of 1.4 mM. All points are the average of triplicate data points. Initial velocities were globally fit to Eq. 3 using Grafit (version 4.0.10).

IC_{50} equation

$$\text{percent inhibition}=100\%/[1+([I]/IC_{50})^S] \qquad \text{(Eq. 1)}$$

IC_{50} to K_i equation for competitive inhibitors (*18*)

$$K_i = IC_{50}/(1+[S]/K_m) \quad \text{(Eq. 2)}$$

Competitive inhibition equation

$$v = V_m[S]/\{K_m(1+[I]/K_{ic}) + [S]\} \quad \text{(Eq. 3)}$$

where IC_{50} is the half maximal inhibitory concentration and S is a slope factor in Eq. 1. K_m is the apparent Michaelis constant for a varied substrate at a fixed concentration of the second substrate and V_m is maximum velocity, S is the varied substrate, and K_{ic} is the competitive inhibition constants.

Virulence phenotype of hsd. We obtained a transposon mutant strain (ST144) with an insert into *hsd (Rv1106c)* from TARGET (Tuberculosis Animal Research and Gene Evaluation Taskforce, NIH/NIAID NO1-AI30036). Complementation of the *hsd* mutant (strain ST160) was carried out by cloning the entire open reading frame of *Rv1106c* with 999 bp upstream of the N-terminus into pMV306. This construct was electroporated into ST144 and selection for Hyg-r transformants yielded strain ST160. Wild-type and mutant cultures were used to infect THP-1 cells that had been differentiated into macrophage-like cells and intracellular growth was monitored for 7 days by plating for cfu (colony forming units). C57BL/6 mice were infected by aerosol with the wild-type strain, the *hsd* mutant strain, and the complemented strain. The growth rate of the bacteria in the lung was assessed for 4 months (*19*). The *hsd* mutant was tested in the guinea pig infection model by NIH/NIAID under the TARGET contract.

High-throughput crystallization screening. The 3β-hydroxysteroid dehydrogenase (*M. tb* Hsd) was purified as described in Chapter 2, and dissolved in 20 mM triethanolamine hydrochloride buffer, pH 8.5 (3.65 mg/mL). Five samples were tested: A,Hsd; B, Hsd with 0.85 mM NAD^+; C, Hsd with 0.85 mM NADH; D, Hsd with 0.85 mM NAD^+ and 0.85 mM androstenedione; and E, Hsd with 0.85 mM NADH and 0.85 mM dehydroepiandrosterone. Initial high-throughput crystallization screening was performed at the Hauptman-Woodward Medical Research Institute in Buffalo, New York,

by using the microbatch-under-oil method (*20-21*). 1536 conditions were applied to each sample. Experiment plates were incubated at 23 ºC and imaged immediately before adding the protein solution and also at the following intervals after addition of the protein solution: one day, one week, two weeks, three weeks, four weeks and six weeks.

III. Results and discussion

Determination of inhibitor K_i values and the mechanism of inhibition against M. tb Hsd. The inhibition potency of 6-azasteroids **1**-**7**, and 4-azasteroids **9**-**10** (i.e., finasteride and dutasteride) were determined by measuring their IC_{50}s (Figure 3-1 and Table 3-1). Trilostane, **8**, was determined to be a 200 μM competitive inhibitor against dehydroepiandrosterone of *M. tb* Hsd (Chapter 2). Furthermore, a full kinetic analysis with both finasteride (4-azasteroid) and GI190639x (6-azasteroid) confirmed a competitive inhibition pattern with respect to steroid substrate (Figure 3-2). 2% DMSO was added to the activity assay as a control and showed no inhibition or activation of the enzyme.

As compared to the 5α-reductase inhibition profile, dutasteride, a much better inhibitor than finasteride, showed no inhibition of *M. tb* Hsd, which fits its selectivity for 5α-reductase over mammalian Hsd. The 6-azasteroids tested show better inhibition of *M. tb* Hsd compared to 4-azasteroids. Their potency against *M. tb* Hsd ranged from 800 nM to 100 μM, which provided a useful distribution to further investigate the structure activity relationship as well as the further inhibitor screen of *M. tb* Hsd. Compounds **5** and **6** showed the best inhibition among all the compounds tested and their structures mimic the product of *M. tb* Hsd. However, their inhibition potency was not high enough to sort out whether this enzyme could be targeted for antibiotic development. Structure-activity

relationship studies and further modification of those compounds for better inhibition against *M. tb* Hsd are required.

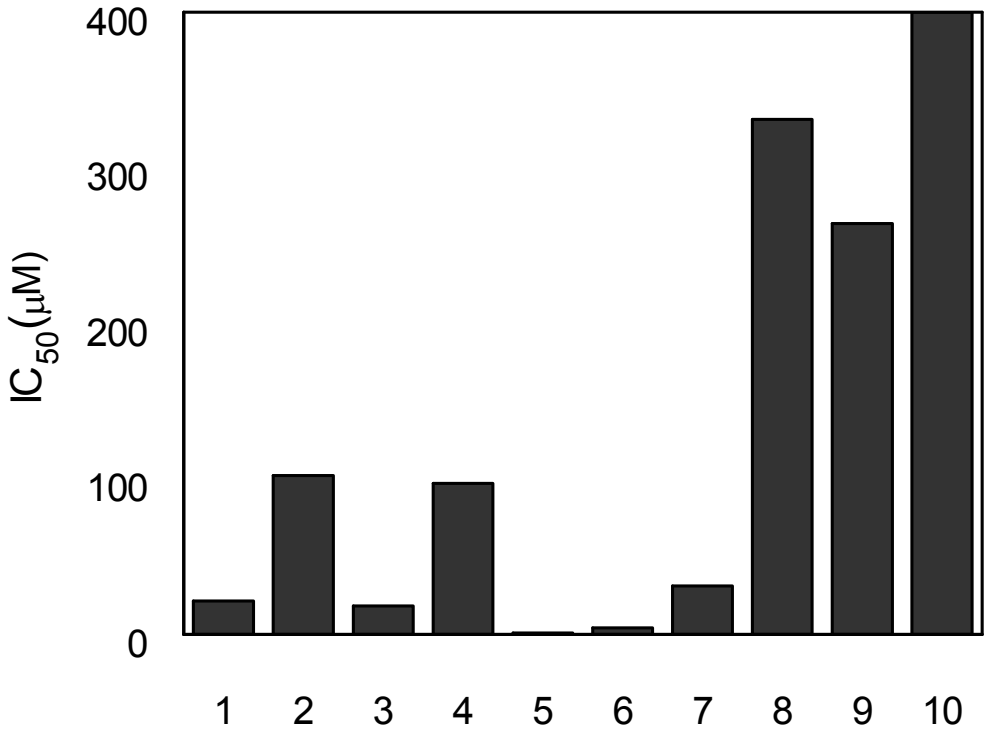


Figure 3-1. Inhibition of $rH_6$3βHSD by 6-azasteroids, 4-azasteroids and trilostane.

Table 3-1. Inhibition of *M. tb* 3βHSD, bovine 3βHSD and 5α-reductases (5AR) by 6- and 4-azasteroids.

#	Lab code or name	IC_{50} with *M. tb* 3βHSD (μm) [a]	K_i with *M. tb* 3βHSD (μm)[a]	K_i with bovine 3βHSD (nM)	K_i with 5AR1 (nM)	K_i with 5AR2 (nM)	Ref.
1	GI171651x	21.5	10.0	500	5.0	<0.1[c]	(*17*)
2	GI203599A	102	47.6	17000	17	26	d
3	GI201208x	18.3	8.5	47	29	0.2	d
4	GI172902x	97.1	45.3	6.1	nd	nd	d
5	GI235875x	0.81	0.38	nd	1.0	2.3	(*11*)
6	GI235971x	4.2	1.96	nd	0.8	7.9	(*11*)
7	GI190639x	31	14.5	1600	8.8	<0.1[c]	(*17*)
8	trilostane	331	154	50	nd	nd	(*22*)
9	finasteride	264	123	11000	150	0.18	(*10*)
10	dutasteride	na[b]	na[b]	nd[e]	6	7	(*12*)

[a]DHEA was fixed at 150 μM and NAD^+ was fixed at 3.5 mM (Conditions: 100 mM TAPS, 150 mM NaCl, 30 mM $MgCl_2$ buffer, pH 8.5, 30 °C). [b]No inhibition activity was observed. [c]IC_{50}. [d]Data provided by GlaxoSmithKline. [e]nd. Not determined.

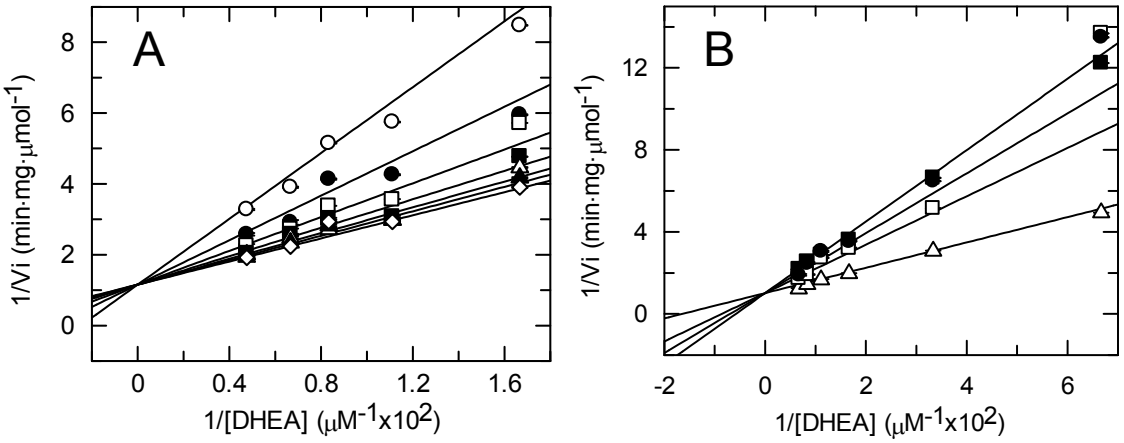


Figure 3-2. Finasteride and GI190639x inhibition kinetics of *M. tb* Hsd. A, Double-reciprocal plot of initial velocity against DHEA at fixed NAD^+ (1 mM). Finasteride concentrations used were: ○, 250 μM; ●, 125μM; □, 62.5 μM; ■, 31.3 μM; ∆, 15.6 μM; ▲, 7.8 μM; and ◊, 0 μM. B, Double-reciprocal plot of initial velocity against DHEA at fixed NAD^+ (1.4 mM). GI190639x concentrations used were: ■, 38.3 μM; ●, 28.7 μM; □, 19.1 μM; and ∆, 0 μM. Conditions: 100 mM triethanolamine hydrochloride buffer, pH 8.5, 30 °C. The data shown are the average of three independent experiments, and the errors are the standard deviation of measurement.

Virulence phenotype of hsd. Three different models were used to elucidate the virulence phenotype of *Rv1106c* (*hsd*). First, the role of Hsd in *M. tb* growth in macrophages was assessed and no difference in intracellular growth rate was detected between wild-type and the *hsd* mutant. Second, the role of the *hsd* mutant in the mouse infection model was tested. The *hsd* mutant's ability to grow and survive in C57BL/6 mice did not diminish compared with that of wild-type *M. tb* after 4 months. Third, the role of the *hsd* mutant in granuloma formation was assessed using the guinea pig infection model, a better model resembling the human disease. No differences between mutant and complemented mutant in growth or in histology were detected (Figure 3-3). Taken together, *hsd* is not required for the *M. tb* virulence, and the inhibitor to the enzyme Hsd

may not lead to a direct effect on *M. tb* virulence. However, Hsd catalyzes the conversion of 3β-hydroxysteroid to 3-ketosteroid, an early step of cholesterol metabolism, and its downstream metabolites could modulate the *M. tb* virulence in an indirect manner. Further analysis of the role of *hsd* (Chapter 4 & 5) will help understand the whole cholesterol metabolic pathway and its function in *M. tb*.

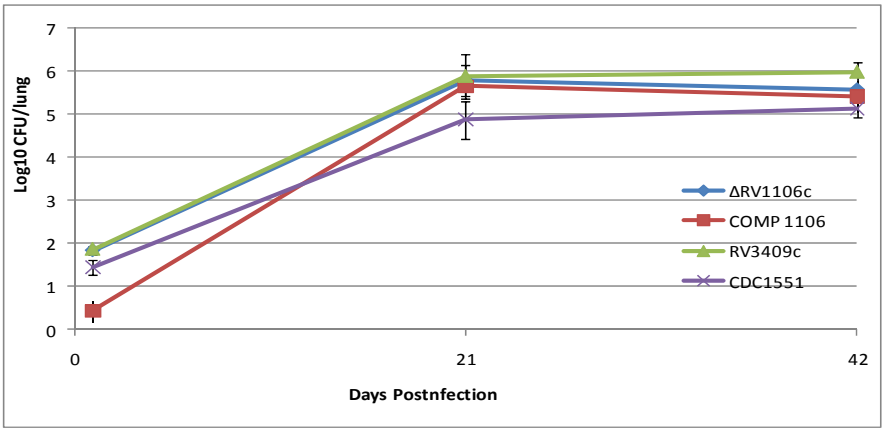


Figure 3-3. Granuloma formation in guinea pigs infected with wild type, the *hsd* mutant, the *hsd* complemented mutant, and the *Rv3409c* mutant. *Rv3409c* encodes a putative cholesterol oxidase in *M. tb*.

High-throughput crystallization screening. Enzymatic kinetic studies of both wild-type *M. tb* Hsd and four mutants have suggested an ordered bi-bi mechanism and a catalytic tetrad of Asn-Ser-Tyr-Lys (Chapter 2). However, the detailed catalytic mechanisms of the oxidation and isomerization steps remain largely unclear, with no information about the active site architecture. Direct structural evidence is needed to resolve these issues. We undertook the preliminary crystallization screening and the potential conditions showing a trend of crystals are summarized in Table 3-2.

Additional trials were done with 7 mg/mL *M. tb* Hsd in 50 mM triethanolamine hydrochloride, pH 8.5, 18 ºC, using the sitting-drop crystallization method. Hampton crystal screen 1 & 2, Optimix 1 and Optimix 3 (Fluidigm Corporation) were used. The

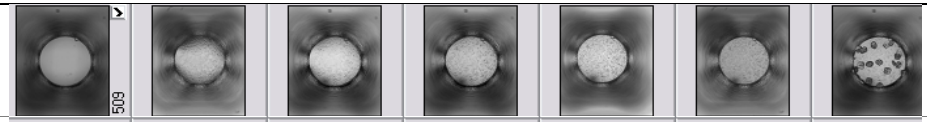
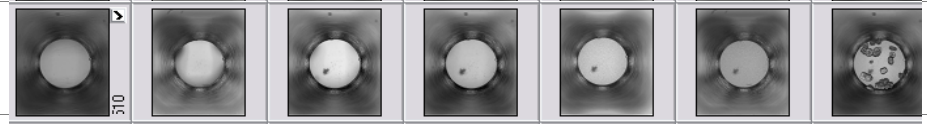
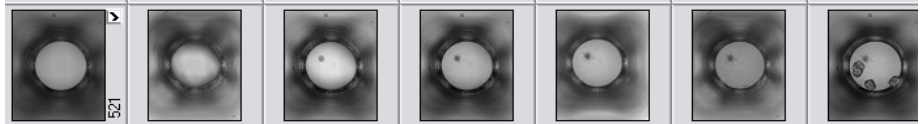
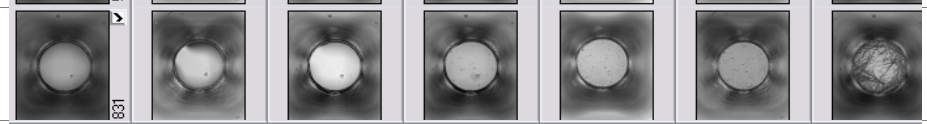
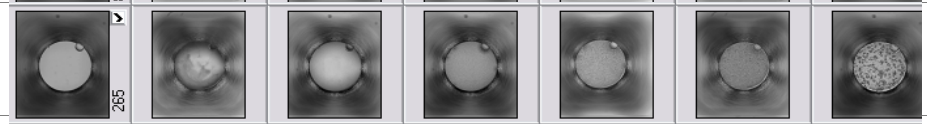
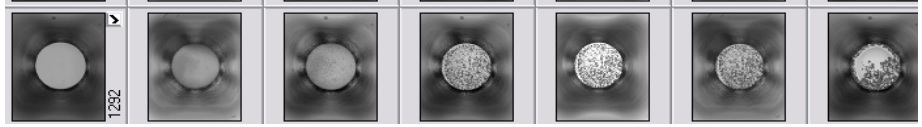
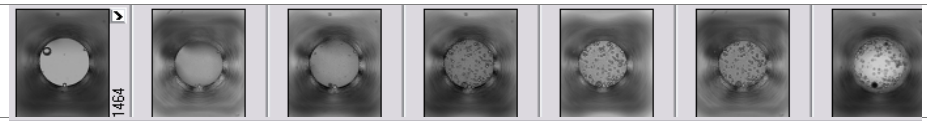
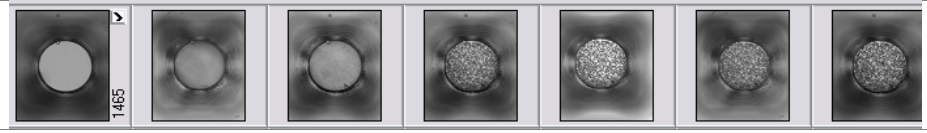
following conditions produced crystals: Hampton crystal screen 1 & 2 E3 (0.4 M ammonium phosphate monobasic), G6 (0.1 M HEPES, pH 7.5, 10% w/v polyethylene glycol 6,000, 5% v/v (+/-)-2-methyl-2,4- pentanediol), and H10 (0.1 M sodium chloride, 0.1 M BICINE, pH 9.0, 20% v/v polyethylene glycol monomethyl ether 550); Optimix 1 C6 (0.25 M sodium tartrate, 0.1 M bis-tris propane, pH 7.0, 4% w/v PEG 10,000, 10% v/v MPD) and D3 (0.5 M sodium acetate, 5% w/v Jeffamine M-600). The current data suggest that solvents at basic pH with detergents are the favored conditions for *M .tb* Hsd crystallization.

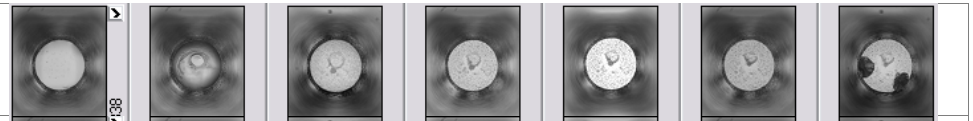
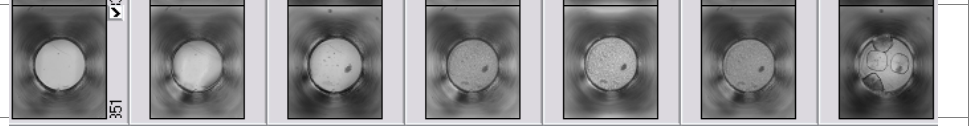
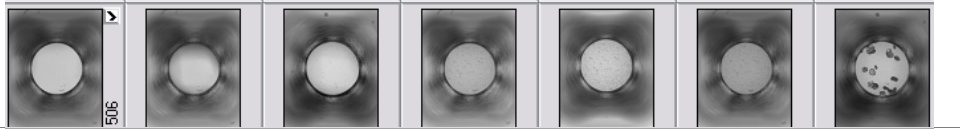
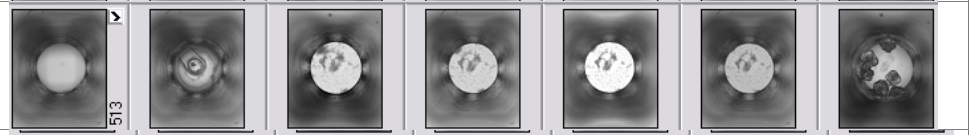
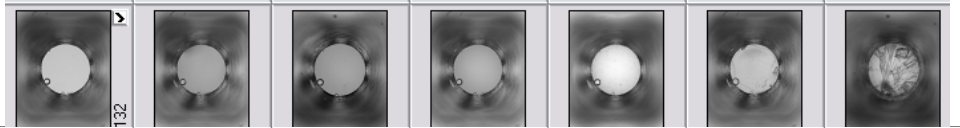
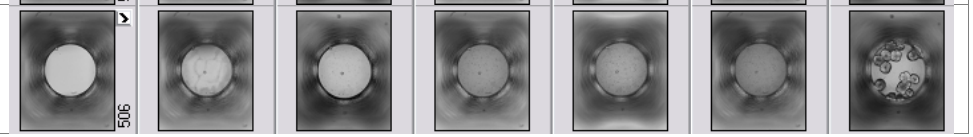
Crystallization of *M. tb* Hsd under the current conditions and those with salts, buffers and precipitants near to the current conditions will be performed using the hanging-drop crystallization method. The crystal structure of *M. tb* Hsd will provide information on the active site and substrate or inhibitor interactions with the enzyme, and will be helpful to fully characterize the detailed catalytic mechanisms.

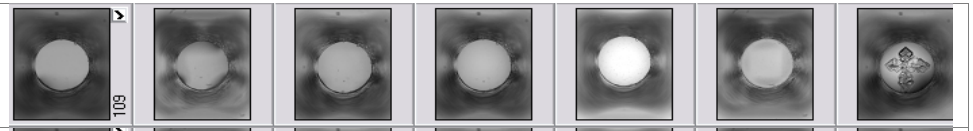
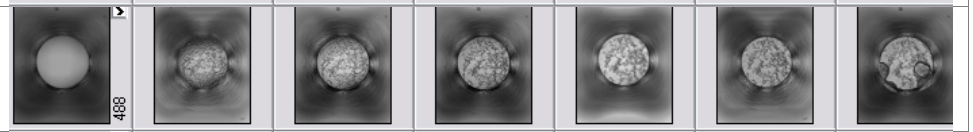
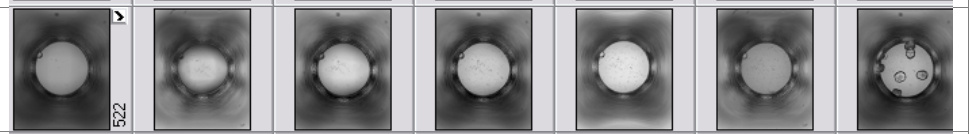
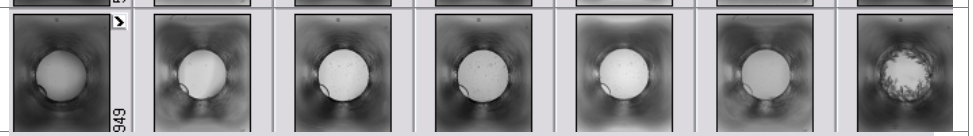
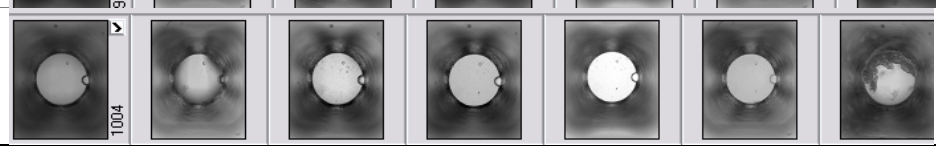
ACKNOWLEDGMENT

We thank Drs. Eugenie Dubnau and Irina Kolesnikova (PHRI, UMDNJ) for performing *M. tb* and animal experiments. We thank TARGET program for guinea pig infection experiments.

Table 3-2. High-throughput crystallization screening results performed at the Hauptman-Woodward Medical Research Institute.

Sample	Conditions	Well#	Images[a]
HSD-2[b]	0.1 M Potassium chloride 0.1 M TAPS, pH 9 40% (w/v) PEG 8000	509	
	0.1 M Potassium chloride 0.1 M CAPS, pH 10 40% (w/v) PEG 8000	510	
	0.1 M Rubidium chloride 0.1 M TAPS, pH 9 40% (w/v) PEG 800	521	
	0. M Lithium sulfate monohydrate 0.1 M CAPS, pH 10 40% (w/v) PEG 1000	831	
	0.2 M Lithium Sulfate monohydrate 0.10 M Tris Hydrochloride, pH 8.5 30% (w/v) PEG 4000	1265	
	0.2 M Magnesium Formate dehydrate	1292	
	0.4 M Magnesium Formate 0.1 M Bis-Tris Propane, pH 7	1464	
	0.4 M Magnesium Formate 0.1 M Tris, pH 8.5	1465	

HSD-3[c]	0.1 M Potassium acetate 0.1 M TAPS, pH 9 24% (w/v) PEG 20000	338	
	0.1 M Potassium thiocyanate 0.1 M CAPS, pH 10 24% (w/v) PEG 20000	351	
	0.1 M Rubidium chloride 0.1 M HEPES, pH 7.5 20% (w/v) PEG 8000	431	
	0.1 Potassium carbonate 0.1 M Tris, pH 8 40% (w/v) PEG 8000	506	
	0.1 M Potassium nitrate 0.1 M HEPES, pH 7.5 40% (w/v) PEG 8000	513	
HSD-4[d]	0.85 M Potassium carbonate 0.1 M CAPS, pH 10	117	
	0.88 M Potassium nitrate 0.1 M CAPS, pH 10	132	
	0.1 M Potassium bromide 0.1 HEPES, pH 7.5 40% (w/v) PEG 8000	502	
	0.1 M Potassium carbonate 0.1 M Tris, pH 8 40% (w/v) PEG 8000	506	

HSD-5[e]	1.33 M Potassium bromide 0.1 M TAPS, pH 9	109	
	0.1 M Lithium bromide 0.1 M TAPS, pH 9 40% (w/v) PEG 8000	488	
	0.1 M Rubium chloride 0.1 CAPS, pH 10 40% (w/v) PEG 8000	522	
	0.1 M Potassium nitrate 0.1 M CAPS, pH 10 20% (v/v) PEG 400	949	
	0.05 M HEPES, pH 6.8 12.5% (w/v) PEG 3350	1004	

[a]Each row represents images taken at 7 time points: 0 day, 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, and 6 weeks (from left to right); [b]HSD-2: HSD with 0.85 mM NAD^+; [c]HSD-3: HSD with 0.85 mM NADH; [d]HSD-4: HSD with 0.85 mM NAD^+ and 0.85 mM androstenedione; and [e]HSD-5: HSD with 0.85 mM NADH and 0.85 mM dehydroepiandrosterone. Incubation temperature was 23 ^oC.

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Chapter 4

Cholesterol metabolism pathway in *Mycobacterium tuberculosis*

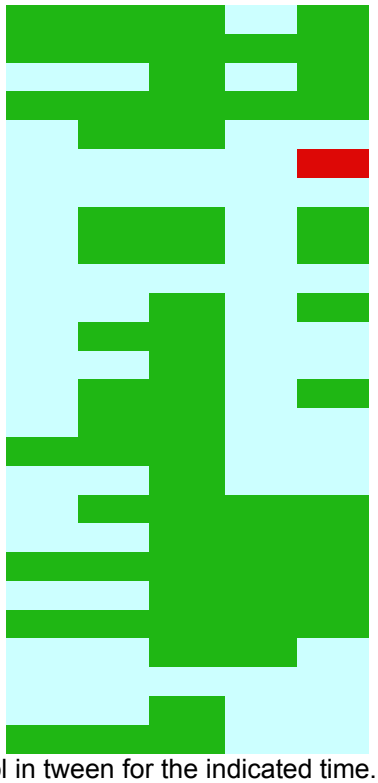
This chapter is partially adapted from a paper that was published in *Infection and Immunity (1)*.

I. Introduction

It has been reported that lipid metabolism plays an important role in *M. tb* infection (*2*) and cholesterol metabolism is required for *M. tb* virulence. Cholesterol is degraded by *M. tb* and metabolites are involved in both catabolic and anabolic pathways (*3*). A group of genes in the actinomycete *Rhodococcus RHA1* were shown to be upregulated during incubation with cholesterol, and were proposed to be responsible for cholesterol catabolism (*4*). An 83-gene region (referred as the "Cho-region") of the *M. tb* chromosome contains many orthologs to the 223-gene region in *Rhodococcus RHA1 (4)* and is proposed to encode proteins that catabolize cholesterol. However, the second cluster of cholesterol-regulated genes (ro6687-ro6698) in *R. RHA1*, proposed to degrade C/D-ring of cholesterol, is not present in *M. tb*. In order to identify *M. tb* genes and enzymes involved in cholesterol metabolism, the *M. tb* cholesterol regulon was studied by transcriptional profiling (*1*). Fifty-two of the *M. tb* genes in the cho-regulon are upregulated by cholesterol, and forty-six genes in this regulon are under control of the KstR transcriptional regulator, which was originally found in *M. smegmatis* as a transcriptional repressor of many genes annotated as involved in lipid metabolism (*5*) (Table 4-1). Although the cholesterol metabolic pathways in *M. smegmatis*, *R. RHA1* and *M. tb* are highly conserved, the KstR regulation and cholesterol regulons are different to some extent. *hsd*, which encodes a 3β-hydroxysteroid dehydrogenase, is repressed by KstR in *M. smegmatis* but not *in M. tb*. Moreover, the *hsd* promoter region is not conserved between *M. tb* and *M. smegmatis***.** The entire Mce4 operon is not upregulated by cholesterol in *M. tb* but is in *R. RHA1.* Further investigation of these genes is needed to fully understand the cholesterol metabolism in *M. tb*.

Table 4-1. "Cho-region" of *M. tb* identified by microarray analysis[a]

M. tb	*Gene name*	*Function*	*c_3*	*c_5*	*c_24*	*k*	*k_c*
*Rv3492c**		CHP MCE associated protein					
*Rv3493c**		CHP MCE associated protein					
*Rv3494c**	*mce4F*	mce4 operon: lipid transfer					
*Rv3495c**	*lprN*	mce4 operon: lipid transfer					
*Rv3496c**	*mce4D*	mce4 operon: lipid transfer					
*Rv3497c**	*mce4C*	mce4 operon: lipid transfer					
*Rv3498c**	*mce4B*	mce4 operon: lipid transfer					
*Rv3499c**	*mce4A*	mce4 operon: lipid transfer					
*Rv3500c**	*YrbE4B/supB*	mce4 operon: lipid transfer					
*Rv3501c**	*YrbE4A/supA*	mce4 operon: lipid transfer					
*Rv3502c**	*hsd4A*	3-ketoacyl-ACP reductase					
*Rv3503c**	*fdxD*	ferredoxin					
*Rv3504**	*fadE26*	Acyl CoA dehydrogenase					
*Rv3505**	*fadE27*	Acyl CoA dehydrogenase					
*Rv3506**	*fadD17*	Fatty acid CoA synthetase					
Rv3507		PE_PGRS					
Rv3508		PE_PGRS					
Rv3509c	*ilvX*	ilvX					
Rv3510c		CHP					
Rv3511		PE_PGRS					
Rv3512		PE_PGRS					
Rv3513c	*fadD18*	Probable fatty acid CoA ligase					
Rv3514		PE_PGRS					
*Rv3515c**	*fadD19*	Fatty acid CoA synthetase					
*Rv3516**	*echA19*	Enoyl-CoA hydratase					
Rv3517	*WhiB3*	transcription factor					
*Rv3518c**	*cyp142*	Cytochrome P450					
*Rv3519**		CHP					
*Rv3520c**		FMN-dependent tetrahydromethanopterin reductase					
*Rv3521**		CHP					
*Rv3522**	*ltp4*	3-ketoacyl-CoA thiolase					
*Rv3523**	*ltp3*	SCPx related 3-ketoacyl-CoA thiolase					
Rv3524		Probable conserved membrane protein					
Rv3525c		Possible siderophore binding protein					
*Rv3526**	*kshA*	Ketosteroid-9a-hydroxylase, oxygenase					
*Rv3527**		CHP					
Rv3528c		HP					
Rv3529c		CHP					
Rv3530c		oxidoreductase					
Rv3531c		CHP					
Rv3532		PPE					
Rv3533c		PPE					
*Rv3534c**	*hsaF*	4-hydroxy-2-oxovalerate aldolase					
*Rv3535c**	*hsaG*	Acetaldehyde dehydrogenase					
*Rv3536c**	*hsaE*	2-Hydroxypentadienoate hydratase					
*Rv3537**	*kstD*	3-Ketosteroid-D1-dehydrogenase					
*Rv3538**	*hsd4B*	2-Enoyl acyl-CoA hydratase					
Rv3539		PE					
*Rv3540c**	*ltp2*	Branched-chain 3-ketoacyl-CoA thiolase					
*Rv3541c**		CHP					
*Rv3542c**		CHP					
*Rv3543c**	*fadE29*	Acyl-CoA dehydrogenase					
*Rv3544c**	*fadE28*	Short/branched chain acyl-CoA dehydrogenase					
*Rv3545c**	*cyp125*	Cytochrome P450 125					
*Rv3546**	*fadA5*	Acetyl CoA acetyltransferase					
*Rv3547**		CHP					
*Rv3548c**		Short-chain dehydrogenase/reductase					

*Rv3549c**		Short-chain dehydrogenase/reductase	
*Rv3550**	*echA20*	Enoyl-CoA hydratase	
*Rv3551**		ATP-dependent CoA-transferase -subunit	
*Rv3552**		ATP-dependent CoA-transferase -subunit	
*Rv3553**		2-Nitropropane dioxygenase	
Rv3554	*fdxB*	fdxB	
Rv3555c		CHP	
*Rv3556c**	*fadA6*	Acetyl-CoA acetyltransferase	
*Rv3557c**		TetR-type transcriptional regulator	
Rv3558		PPE	
*Rv3559c**		Short chain dehydrogenase/reductase	
*Rv3560c**	*fadE30*	Acyl-CoA dehydrogenase	
*Rv3561**	*fadD3*	Acyl-CoA synthetase [AMP forming]	
*Rv3562**	*fadE31*	Acyl-CoA dehydrogenase	
*Rv3563**	*fadE32*	Short/branched chain acyl-CoA dehydrogenases	
*Rv3564**	*fadE33*	Short/branched chain acyl-CoA dehydrogenases	
Rv3565	*aspB*	Possible aspartate aminotransferase	
Rv3566c	*nhoA*	Arylamine N-acetyltransferase	
*Rv3567c**	*hsaB*	3-HSA hydroxylase, reductase	
*Rv3568c**	*hsaC*	2,3,dihydroxybiphenyl 1,2 dioxygenase	
*Rv3569c**	*hsaD*	4,9-DSHA-hydrolase	
*Rv3570c**	*hsaA*	3-HSA hydroxylase, oxygenase	
*Rv3571**	*kshB*	Ketosteroid-9a-hydroxylase, reductase	
Rv3572		CHP	
*Rv3573c**	*fadE34*	Acyl-CoA dehydrogenase	
*Rv3574**	*kstR*	TetR regulator	

[a] Cells were grown into mid-log phase and induced with cholesterol in tween for the indicated time. In the shaded columns, green shading indicates at least 1.5-fold induction, red indicates at least 1.5 decrease in expression, and blue shading indicates no change in induction by: c3, cholesterol in *M. tb* after 3 h; c24, cholesterol in *M. tb* after 24 h; k, mutation of *kstR* in *M. tb* and kc, mutation of *kstR* in *M. tb* induced with cholesterol. White indicates missing data. CoA, Coenzyme A, CHP, conserved hypothetical protein, HP, hypothetical protein. *Denotes genes that have orthologs in *Rhodococcus* "Cho-region." [modified with permission from *(1)*; copyright 2009, American Society for Microbiology].

M. tb can use cholesterol as a sole carbon source (*3*), and the growth defects of some mutants indicate that those genes are required for the cholesterol metabolism. Among those genes, the Mce4 transporter (*Rv3499c-Rv3494c*) was first identified to encode a cholesterol import system (*3*). The *igr* operon (*Rv3540c-Rv3545c*), containing a cytochrome P_{450} (*cyp125*), acylCoA dehydrogenases (*fadE28* and *fadE29*), enoylCoA hydratase (*Rv3542c* and *Rv3541c*), and a lipid transfer protein (*ltp2*), has also been shown to be important for the intracellular growth of *M. tb* (*6*). The deletion of this operon led to a growth defect in the presence of cholesterol (*7*). Three ring-degradation genes, Hsd (*Rv1106c*, 3β-hydroxysteroid dehydrogenase), HsaA (*Rv3570c*, 3-HSA hydroxylase) and HsaC (*Rv3568c*, 2,3-dehydroxyphenyl dioxygenase) were identified to be required

for the growth on cholesterol as a sole carbon source (*8*) (Dubnau, unpublished/pers. comm.). Some of the β-oxidation genes induced by cholesterol and repressed by *kstR*, including *echA19* (*Rv3516*), *fadD19* (*Rv3515c*), *fadA5* (*Rv3546*), and *fadE29* (*Rv3543*) were also monitored. Only the *fadA5* and *fadE29* mutants were unable to grow on cholesterol as a sole carbon source (Dubnau, unpublished/pers. comm.).

To assess the importance of genes for *M. tb* infection, wild-type strains and mutants were tested during mouse infection. The Mce4 operon was required for the *M. tb* survival in the mouse spleen (*9*) and mouse lung (*3*), especially at a later stage of infection. Similarly, the *fadA5* mutant was attenuated in the mouse lung infection. The *fadA5* mutant initially grew as rapidly as the wild-type strain and the number of colony forming units in the mouse lungs decreased about 10-fold compared with the wild-type strain at about 8 weeks (*1*), indicating that *fadA5* is only attenuated in the persistent stage of infection. Strains with mutations in *hsaC*, *fadE29 and the whole igr operon* were attenuated, having a slow growth phenotype from the start of infection (*8, 10*) (Dubnau, pers. comm.). Strains with mutations in *echA19, hsaA,* or *fadD19* were not attenuated within 13 weeks after infection (Dubnau, pers. comm.).

To further investigate the cholesterol metabolic pathway, we analyzed the metabolites of *hsd* and another five genes induced by cholesterol and repressed by *kstR* (*fadA5, hsaA, echA19, fadD19*, and *fadE29*). Liquid chromatography coupled with mass spectrometry (LC/MS) has been used as a routine technique to decipher metabolism (*11*). We used LC/MS combined with radioactive and stable isotope labeled substrates in our studies.

II. Materials and methods

Materials. Cholesterol, cholest-4-en-3-one, cholest-5-en-3-one, NAD^+, dehydroepiandrosterone, pregnenolone, hydrocortisone, corticosterone, cortisone, 4-androsterone-3,17-dione were purchased from Sigma-Aldrich (St. Louis, MO). TritonX-100 and progesterone were from Aldrich Fine Chemical Co. (Milwaukee, WI). Fludrocortisone was from Waterstone Technology (Indianapolis, IN). [4-^{14}C]Cholesterol was purchased from PerkinElmer (Waltham, MA). Cholesterol-2,2,3,4,4,6-d_6 and cholesterol-25,26,26,26,27,27,27-d_7 were from C/D/N Isotopes Inc.(Quebec, Canada).

Bacterial strains, media and growth conditions. M. tb cultures were grown at 37 °C in Middlebrook 7H9 liquid media (Becton Dickinson), supplemented with 0.05% Tween-80, 10% albumin-dextrose-NaCl complex (ADN) (*12*) and 0.2% glycerol, or on Middlebrook 7H10 plates supplemented the same way. Kanamycin was added at 20 μg ml^{-1} and hygromycin at 100 μg ml^{-1}. When cholesterol was used as an inducer for metabolic analysis, cultures were grown to mid-log phase in supplemented 7H9 and then cholesterol (1 mg ml^{-1} final concentration) in Tween-80 (1% w/v final concentration) was added and the cultures were incubated for an additional 30 min to 48 h. Cholesterol stock solutions (20 mg ml^{-1}) were prepared in aqueous Tween-80 (20% w/v). Treatment with 1% Tween-80 only was used as the control. Cultures were harvested by centrifugation. The cell pellets were autoclaved and the culture filtrate was collected and sterile filtered for further analysis.

Cloning and construction of M. tb mutant strains. DNA recombinant techniques were performed following standard procedures (*13*). All restriction enzymes and modifying enzymes were commercially obtained from Promega Laboratories or New England Biolabs and used according to the manufacturer's recommendations. Electroporation of *M. tb* and DNA Southern blot analysis from the *M. tb* chromosome

were performed as previously described (*1, 14*). The *fadA5* mutation in *M. tb* was constructed using pSM270, as described (*14*). The *echA19* mutation in *M. tb* was constructed using the temperature-sensitive phage delivery system as described (*15*). DNA fragments flanking *echA19* were prepared by PCR reactions from *M. tb* chromosomal DNA, using DNA primers (Integrated DNA Technologies) and the Fail Safe PCR system (Epicenter Biotechnologies). The PCR-generated DNA molecules were ligated to PCR Blunt II Topo Vector (Invitrogen), using manufacturer's protocols and the cloned inserts sequenced using M13 forward and reverse primers prior to subcloning into pJSC284 (*15*). The resulting plasmid construct containing DNA fragments separated by the Hyg cassette of pJSC284 was then used to construct the temperature-sensitive transducing phage for transduction to *M. tb* H37Rv. Southern blot analysis (data not shown) of presumptive recombinants from both experiments indicated that strains had the predicted disruptions in *fadA5* (strain ST76) and *echA19* (strain ST67). Complementation of the *fadA5* mutant was accomplished by cloning the entire open reading frame with 166 bp flanking at the N-terminus into the integrative plasmid pMV306 (MedImmune, Inc.) that confers hygromycin resistance. This construct was electroporated into ST76 and selection for Hyg-r transformants resulted in strain ST93. Complementation of the *hsd* mutant was carried out by cloning the entire open reading frame of *Rv1106c* with 999 bp flanking at the N-terminus into pMV306. This construct was electroporated into ST144 and selection for Hyg-r transformants yielded strain ST160. We obtained transposon mutant strains with inserts into *hsd, choD, fadE29*, *fadD19*, *mmpL5* and *hsaA*, from TARGET (Tuberculosis Animal Research and Gene Evaluation Taskforce, NIH/NIAID NO1-AI30036).

*Lipid extractions and LC/MS Analysis***.** Cell pellets were extracted by the Bligh-Dyer method (*16*), concentrated to dryness and resuspended in EtOAc. The culture supernatants were extracted with EtOAc twice. The aqueous layers were acidified to pH

5 and extracted with EtOAc twice. For all samples, the EtOAc extracts (neutral or acidified) were washed with H_2O three times, concentrated, and the concentrates were analyzed by LC/MS/UV. A Waters ACQUITY Ultra Performance LC system (Milford, MA), equipped with a PDA detector and a SQ detector was used for identification of metabolites. Chromatography was performed with a C18 reversed-phase column (2.1mm x 100mm, i.d., 1.7 μm particle size, Waters ACQUITY UPLC BEH) maintained at 55 °C. The elution solvents were A: 10% MeOH in H_2O and B: MeOH, and the flow rate was 0.5 ml min^{-1}. The eluting solvent was isocratic for 0.02 min with 100% A, followed by a linear gradient to 44% A in 0.3 min, a second linear gradient to 0% A in 2 min, isocratic for 7 min with 0% A, a third linear gradient to 100% A in 0.5 min and isocratic with 100% A for 0.5 min. The mass spectrometer was operated with an atmosphere pressure chemical ionization source in positive ion mode with the source temperature set to 150 °C, the desolvation temperature was set to 450 °C with a corona voltage of 1.5 kV and a cone voltage of 30 V. Alternative ionization used was electrospray ionization in positive ion mode with the source temperature set to 120 °C, the desolvation temperature was set to 350 °C with a capillary voltage of 3 kV and a cone voltage of 30 V. The UV detection wavelength range was 200 to 400 nm. Radioactive samples were only analyzed by UV detection. Fractions were collected every 10 sec and analyzed by liquid scintillation counting after addition of 4 ml of Scintiverse II LSC cocktail. [2,2,3,4,4,6-^{2}H]-Cholesterol or [25,26,26,26,27,27,27-^{2}H]-cholesterol were used for deuterium labeling experiments.

Cell lysate analysis. The pellet was resuspended in 50 mM sodium phosphate buffer, pH 7, and the cells were lysed by bead-beating twice for 1 min, with incubation on ice for 2 min between each bead beating. The cell lysate was centrifuged to remove the cell debris and the supernatant was sterile filtered. Lysates were incubated with 150 μM cholesterol or dehydroepiandrosterone and 2.8 mM NAD^+ in 100 mM TAPS

hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5, at 30 °C for 72 h. The crude protein concentrations of lysates were measured by Bradford protein assay.

Samples were extracted by C18 solid phase extraction. The C18 cartridges (1 mL, Restek, PA) were conditioned with 500 µL methanol and 500 µL H_2O by centrifuging at 1k for 1 min. Samples were loaded on the column and centrifuged at 1.5k for 1 min. Then the columns were washed with 500 µL of water by centrifuging at 1.5k for 1 min and dried by centrifuging for an additional 1 min at the same speed. 500 µL methanol: isopropanol (9:1) was finally applied twice to the column to elute the analytes. The extracts were further analyzed by LC/MS (*vide supra*).

Purification and characterization of a cholesterol-derived apolar lipid from M. tb cells. Cell pellets were extracted by the Bligh-Dyer method (*16*) (*vide supra*). The extracts were concentrated and purified on a preparative TLC plate, developed with a mixture of petroleum ether: EtOAc (4:1). The purity of the isolated fraction was confirmed by LC/MS/UV. ^{1}H NMR, ^{13}C NMR, HMBC, HSQC and $^{1}H^{1}H$ COSY spectra were recorded with a Bruker Avance 700 NMR Spectrometer. The MS^2 spectrum was obtained with an LTQ-Orbitrap spectrometer (Thermo Scientific). The IR spectrum was recorded with a Nicolet iS10 Series FTIR spectrometer (Thermo Scientific).

III. Results and discussion

Analysis of steroids with LC/MS. A steroid mixture containing 9 standards: cortisone, hydrocortisone, corticosterone, 4-androsterone-3,17-dione, dehydroepiandrosterone, progesterone, pregnenolone, cholest-4-en-3-one, cholesterol, was separated well (Figure 4-1) under gradient conditions using methanol/water and ESCi (multi-mode ionization source with API and ESi) positive mode as described in the Materials and Methods section. Fifty picograms of each steroid was injected and standard curves were generated under SIR (selected ion recording) mode. API

(atmospheric pressure ionization) mass spectra of steroids containing a conjugated 3-one and 4-ene groups were characterized by a dominant MH^+ with little fragmentation. API mass spectra of steroids containing a hydroxyl group resulted in an MH^+ with loss of one water molecule, with a detection limit of approximately 10-fold lower than the conjugated compounds. Methanol is a better solvent for protonation, and the addition of acetic acid helps the sensitivity, with the same pattern observed by Ma and Kim (*17-18*). However, due to the pH sensitivity of some samples, water/methanol was used in the rest of the LC/MS assays. All the LC/MS experiments performed with *M. tb* samples are summarized in Table 4-3.

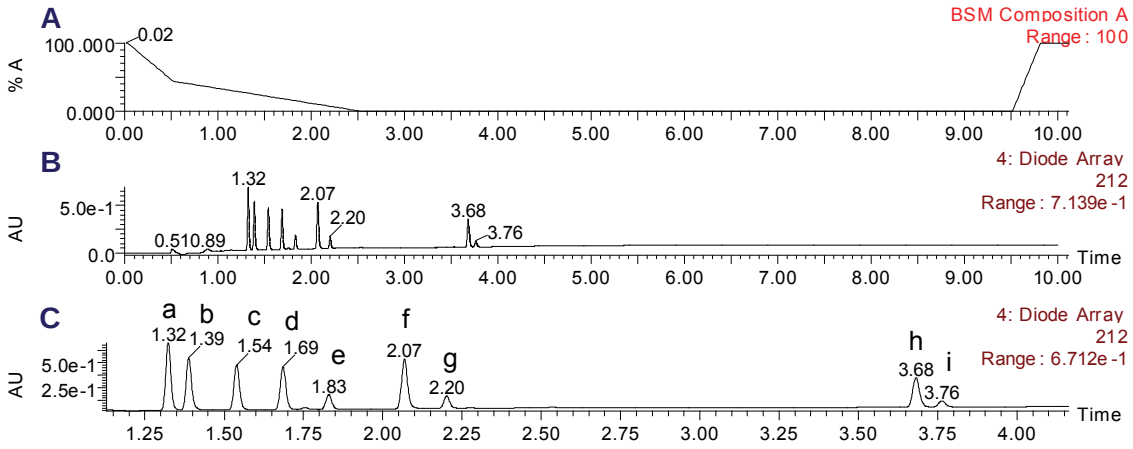


Figure 4-1. LC/MS standard curve of nine steroids detected at 212 nm. (A) The elution gradient. Solvent A: 10% methanol in water, and solvent B: methanol. (B) The chromatogram with detection at 212 nm. (C) The expanded view of (B) from 1.15 to 4.15 min is shown. The peaks a-i represent cortisone, cortisol, corticosterone, 4-androstene-3,17-dione, dehydroepiandrosterone, progesterone, pregnenolone, cholest-4-en-3-one, and cholesterol, respectively.

Time course of cholesterol metabolism by M. tb. In culture, *M. tb* metabolism of cholesterol is both anabolic and catabolic. A more detailed analysis of cholesterol metabolism by wild-type *M. tb (CDC1551)* was performed in order to determine the time scale and which intermediates are accumulated in the pathway. Wild-type *M. tb* culture in standard medium was incubated with 1 g/liter cholesterol for 5 hours to induce

metabolic activity. Then [4-^{14}C]-cholesterol was added and aliquots removed at 0.5, 1, 2, 5 and 20 h post-addition of radioactive tracer. Cells were collected and extracted with chloroform/methanol. Little to no radioactivity remained in the culture filtrates indicating that the cholesterol is rapidly transported into the bacteria. The cell extracts were analyzed by reversed-phase UPLC, UV/vis detection and scintillation counting (Figure 4-2). Identical samples were prepared without radioactive label for LC/MS/UV analysis to further determine the structure of metabolites. At 0.5 hour, most of the cholesterol had been consumed and the majority of the radioactivity appeared at the early retention times, indicating the formation of intermediates with increased hydrophilicity, e.g. intermediates with degradation of the side-chain. From 0 to 2 hours, the mass balance based on total counts recovered was constant. At 20 hours, half of the mass had been lost, presumably to volatile species like CO_2. The more hydrophobic fractions shown at later time points may represent the lipids with incorporation of ^{14}C. However, the other wild-type *M. tb* (H37Rv) used in this study showed much slower metabolic dynamic properties compared to CDC1551. At 24 hours, only 20% of cholesterol was transported into the cells, and at 48 hours less than 70% of cholesterol was transported.

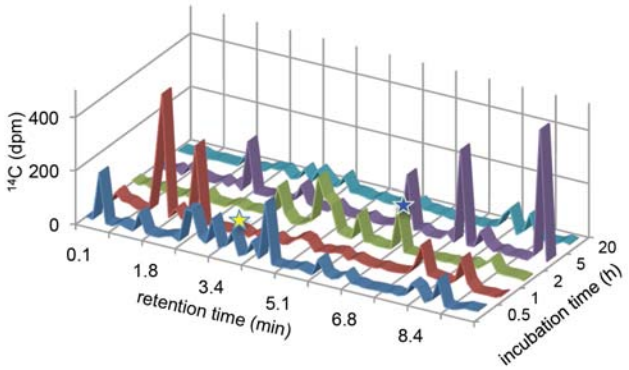


Figure 4-2. Three-dimensional plot of wild-type *M. tb* (CDC1551) cholesterol metabolism time course. Extracts of *M. tb* were analyzed by LC using C18 column, fractions were collected every 10 sec, and counts per fraction were determined by liquid scintillation counting. The yellow star marks the cholesterol peak eluting at 3.7 min. The blue star marks the wax ester peak eluting at 6.7-7 min (*vide infra*).

Hsd is the enzyme responsible for 3β-hydroxysteroid oxidation in M. tb. Gene mining of the *M. tb* genome reveals two putative cholesterol oxidizing enzymes (encoded by *Rv3409c* and *Rv1106c). hsd* (*Rv1106c)* is required for growth on cholesterol as a sole carbon source, while *Rv3409c* is not. To further identify whether *Rv3409c* or *Rv1106c* is the gene responsible for 3β-hydroxysteroid oxidation in *M. tb,* both *in vitro* and *in vivo* experiments were performed. First, *M. tb* cell lysate supernatants were incubated with cholesterol for 72 h, and cholesterol oxidation activities were determined using LC/MS (Figure 4-3A). That is, the amount of cholest-4-en-3-one formed was quantified by the comparison with standard curves and the activity (product formed per min per total protein) was calculated. However, due to the lack of internal standards and the calculation with the amounts of total proteins, the results can only be considered qualitatively. The strains were: wild-type CDC1551, *hsd* (*Rv1106c*, encoding a 3β-hydroxysteroid dehydrogenase), and two *hsd* complemented strains #1 and #2. Disruption of the *Rv1106c* gene fully reduced the cholesterol oxidizing activity of *M. tb*, and this activity was restored in the complemented strains. The *Rv3409c* gene does not compensate for the loss of *hsd* in converting cholesterol to cholest-4-en-3-one, suggesting that *hsd* is the only 3β-hydroxysteroid oxidizing gene in *M. tb*. Furthermore, the cholesterol oxidizing activities were only observed in the cell lysate supernatant, confirming that Hsd is expressed intracellularly (Chapter 2). We reasoned that Hsd is the enzyme responsible for 3β-hydroxysteroid oxidation in *M. tb*.

Three other strains, wild-type H37Rv, *fadA5* (encoding a putative acetoacetyl thiolase), and *echA19* (encoding a putative enoyl-CoA hydratase), were also tested for their lysate activities. Both mutants gave similar activity of the conversion from cholesterol to cholest-4-en-3-one as shown in wild-type, indicating that enzymes encoded by those two genes are not involved in the first step of cholesterol metabolism and their possible functions in the side-chain degradation will be discussed (*vide infra*).

Second, the whole cell metabolism of cholesterol by wild-type, the *hsd* mutant, and the *hsd* complemented strains was analyzed (Figure 4-3B). *M. tb* grown in the standard medium was incubated with cholesterol for 5 hours. Extracts were analyzed in the same manner as the samples in Figure 4-2. Accumulation of ^{14}C-cholesterol (~40% of the total counts injected), was observed in the *hsd* mutant (Figure 4-3B). No cholest-4-en-3-one was formed in the *hsd* mutant as determined by the lack of absorbance at 240 nm of the ^{14}C-cholesterol peak at 3.7 min. The LC/MS analysis of the identically prepared non-radioactive samples confirmed this result. Cholesterol oxidation activity was restored in the complemented strain. In addition, the peaks between 1-3.5 min have masses (e.g. m/z of 385 or 399 Th) and retention times consistent with oxygenated cholesterol. And the peaks around 6 to 10 min account for 40% of the total ^{14}C masses. A waxy ester, previously observed in wild type (Figure 4-2), was also produced in the *hsd* mutant with the same mass and retention time, with only 2% of the total counts after 5 hours (*vide infra*). Based on all these experiments, we conclude that Hsd is the only enzyme responsible for 3β-hydroxysteroid oxidation in *M. tb*.

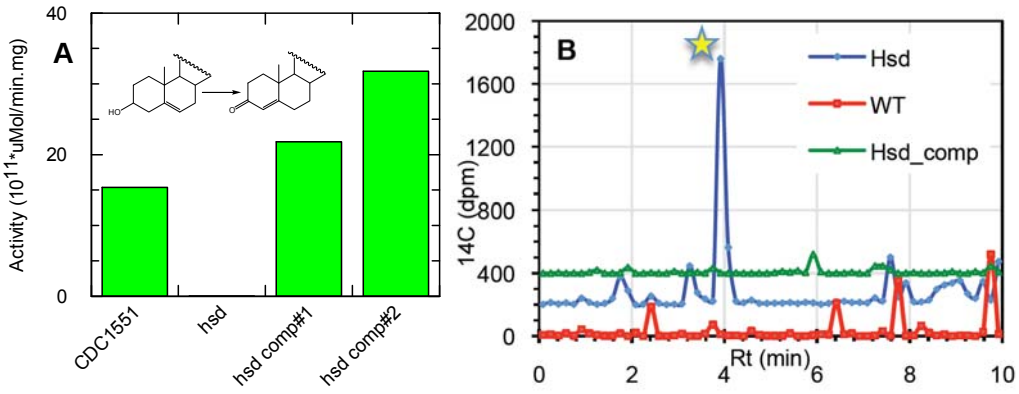


Figure 4-3. (A) Specific activities in cell lysates of uninduced wild-type CDC1551, *hsd* mutant and complemented mutants. (B) LC/UV analysis of wild-type, *hsd* mutant, and *hsd* mutant complemented with *hsd M. tb*, incubated with [4-^{14}C]-cholesterol for 5 h. Chloroform:methanol extracts of *M. tb* were analyzed by LC, and were collected every 10 sec, and counts per fraction were determined by liquid scintillation counting. The peak eluting at 3.7 min and marked with a yellow star contains only cholesterol and has no absorbance at 240 nm, indicating the absence of cholestenone. Data shown are representative of three independent experiments.

Further analysis of M. tb lysate incubated with dehydroepiandrosterone. M. tb lysates from cholesterol-induced or uninduced wild-type and mutant strains were incubated with dehydroepiandrosterone (DHEA), NAD^+, and ATP, at pH 8.0 for 72 h. All samples were extracted with methanol and isopropanol with C18 cartridges and analyzed by LC/MS. Although residues of cholesterol were carried through from the cell cultures into extracts from cholesterol-induced cultures, no metabolites directly produced from cholesterol itself were found. Different products accumulated, from DHEA appeared at 1.5 min to 2 min in the chromatogram (Figure 4-4). There are four main peaks in the chromatograms: unknown peak 1 at 1.62 min, with m/z 287.2, one possible structure is hydroxyl-DHEA ($[M+1-H_2O]^+$); peak 2 at 1.74 min, with m/z 287.2 ($[M+1]^+$) and an absorbance at 240 nm , which is identified as androst-4-en-3,17-dione (AD) by comparing with the standard; peak 3 at 1.80 min with m/z 271.2 ($[M+1-H_2O]^+$), which is identified as intact dehydroepiandrosterone (DHEA); and unknown peak 4 at 1.87 min with m/z 253.3, 271.3, and 343.2. The different products produced by the same mutants induced or uninduced by cholesterol are presumably due to the different protein levels affected by cholesterol. However, under the current conditions, there is no clear trend of products considering the possible functions of those mutants, which may due to the stability of proteins in the lysates or the incubation conditions.

Wild-type lysates were further incubated with steroids (cholesterol, dehydroepiandrosterone, and pregnenolone) in the presence of NAD^+, ATP, and CoA at pH 8.0 for 72 h. Both 4-en-3-one and dien-3-one products of dehydroepiandrosterone and pregenolone were observed, however no dien-3-one form of cholesterol was found in the LC/MS trace. We hypothesized pregnenolone and DHEA are substrates for the first two enzymes, Hsd and KstD, but do not react further, suggesting that they are not substrates for kshA/B under the current conditions. No dien-3-one product detected with cholesterol as substrate may be explained by the fast metabolism of cholesterol

compared to the other two substrates without the C17 side chains. However, the reproducibility was not satisfactory with *M. tb* lysate samples and further metabolism experiments were carried out with whole cells.

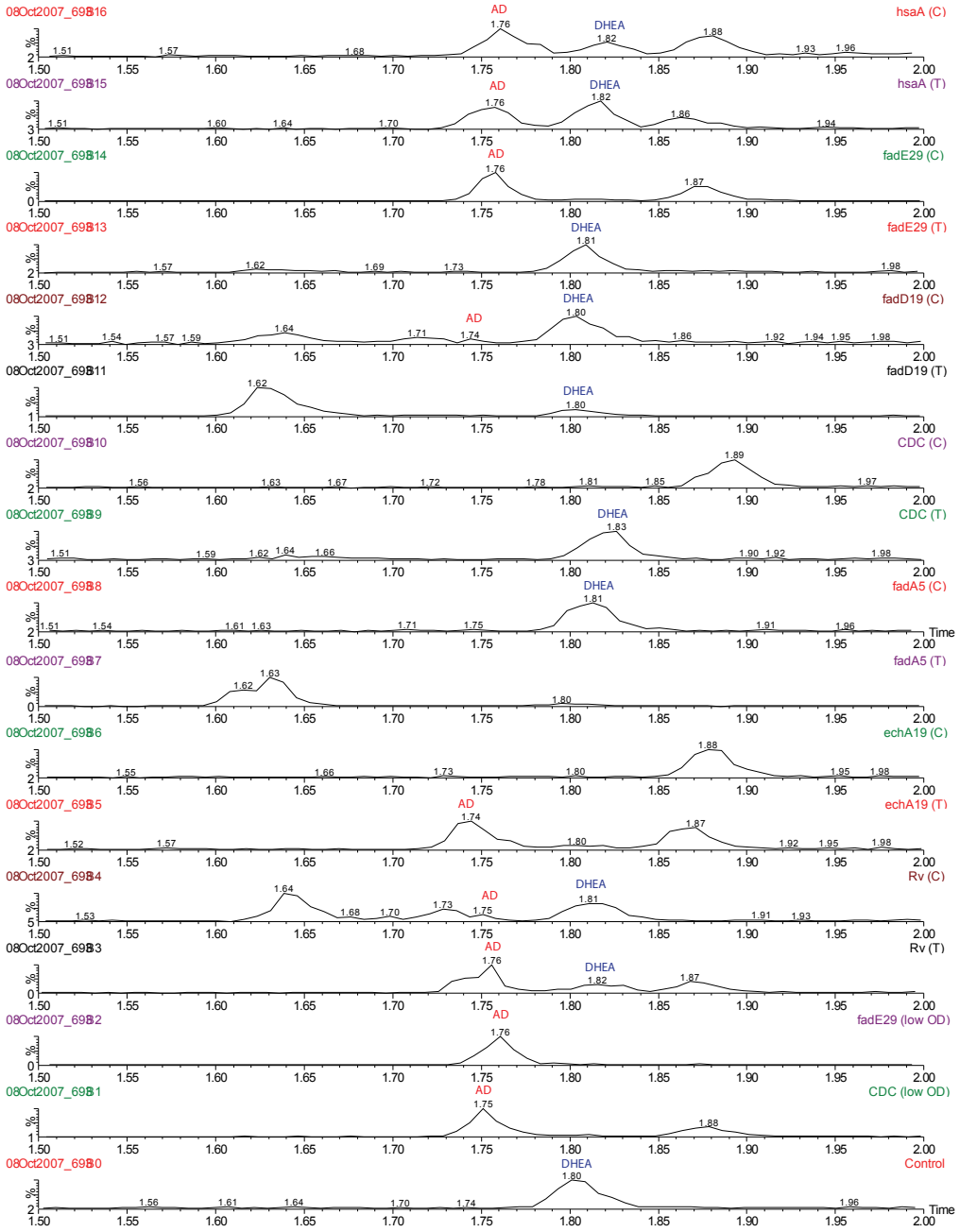


Figure 4-4. Liquid chromatography scanning APCI from 150 – 450 Th in positive ion mode analysis of CDC1551, H37Rv, *echA19*, *fadA5*, *fadD19*, *fadE29* and *hsaA* induced by cholesterol (labeled with C) or grown in Tween-80 (labeled with T) lysates activities. Lysates were incubated with dehydroepiandrosterone, NAD^+, and ATP, at pH 8.0 for 72 h. Dehydroepiandrosterone (DHEA) and androst-4-en-3,17-dione (AD) are labeled on the peaks. Control was prepared without the addition of lysate.

M. tb H37Rv metabolizes cholesterol to androst-4-ene-3,17-dione and androsta-1,4-diene-3,17-dione and the fadA5 mutant is deficient in this activity (1). We grew cultures of H37Rv, the *fadA5* mutant and the complemented *fadA5* mutant in complete medium to the logarithmic stage of growth. Cholesterol was added to the cultures and an aliquot was removed from each to which [4-^{14}C]-cholesterol was added and samples were withdrawn from the radioactive cultures up to 48 hours. The cultures with no [4-^{14}C]-cholesterol were collected 48 hours after addition of cholesterol, lipids of both cell pellets and culture supernatants were extracted, and the extracts were analyzed by LC with MS and UV detection. The wild-type and mutant extracts of cell pellets contained the same metabolites (Figure 4-5). In contrast, the wild-type strain secreted two metabolites into the culture supernatants that were absent in the *fadA5* mutant cultures (Figures 4-6). The two metabolites retained the ^{14}C-label from cholesterol, had λ_{max}'s of 245 and 243 nm, and had MH^+ parent ions at 285.2 and 287.2, respectively (Figure 4-7). These spectroscopic data as well as the retention times were consistent with assignment to predicted metabolites androsta-1,4-diene-3,17-dione (ADD) and androst-4-ene-3,17-dione (AD). Coinjection of the samples with authentic AD confirmed the identity of the second metabolite. Complementation of the *fadA5* mutant strain restored production of ADD and AD (Figure 4-6).

The cell filtrate metabolites from the other wild type strain (CDC1551) were analyzed. However, no AD or ADD was accumulated after 48 hours. This may be because the cholesterol metabolism of this strain is faster than H37Rv or the amount produced is under detection limit. In addition, the *hsd* mutant produces AD and ADD, but at least 1000-fold less than the amount detected in *M. tb* H37Rv. The metabolites of earlier time points in the wild-type and the other mutants are being analyzed to further clarify the functions of these metabolites.

LC/MS analysis of extracts from the supernatants of cultures of the *fadA5* mutant incubated with cholesterol deuterated only in the side chain or only in the A/B rings indicated that the cell culture supernatant contains a complex mixture of cholesterol-derived metabolites that have undergone A/B ring degradation (no deuterium label retained) and that retain part of the deuterium label from the cholesterol side chain (Figure 4-8). We conclude that *fadA5* may be involved in side chain degradation and not ring cleavage of cholesterol.

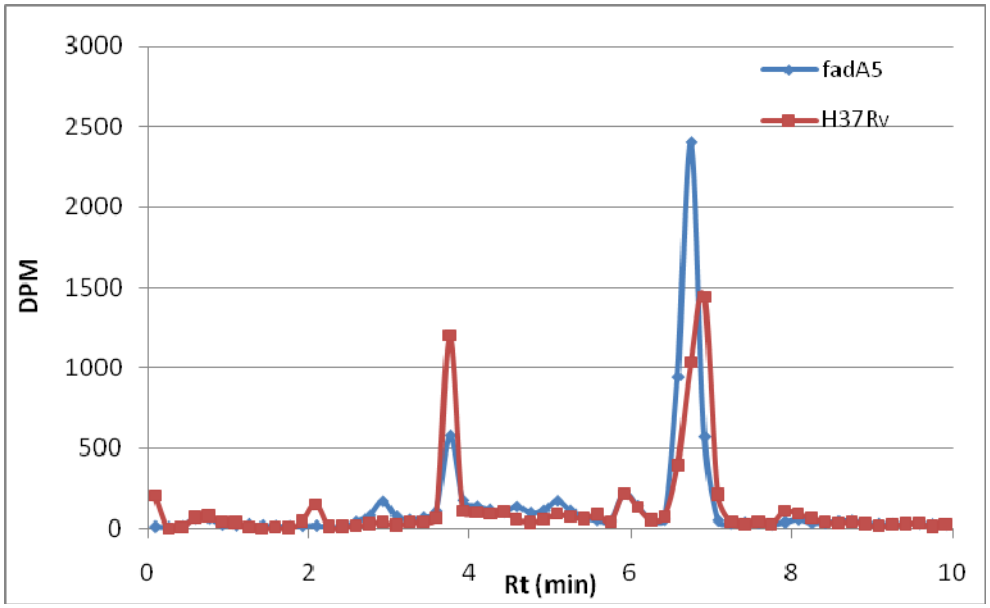


Figure 4-5. LC/UV analysis of wild-type H37Rv and *fadA5* mutant with [4-^{14}C]-cholesterol for 24 h. Chloroform: methanol extracts of *M. tb* cells were analyzed by LC, and were collected every 10 sec, and counts per fraction were determined by liquid scintillation counting. The peak eluting at 3.7 min contains cholesterol and cholest-4-en-3-one. Data shown are representative of three independent experiments.

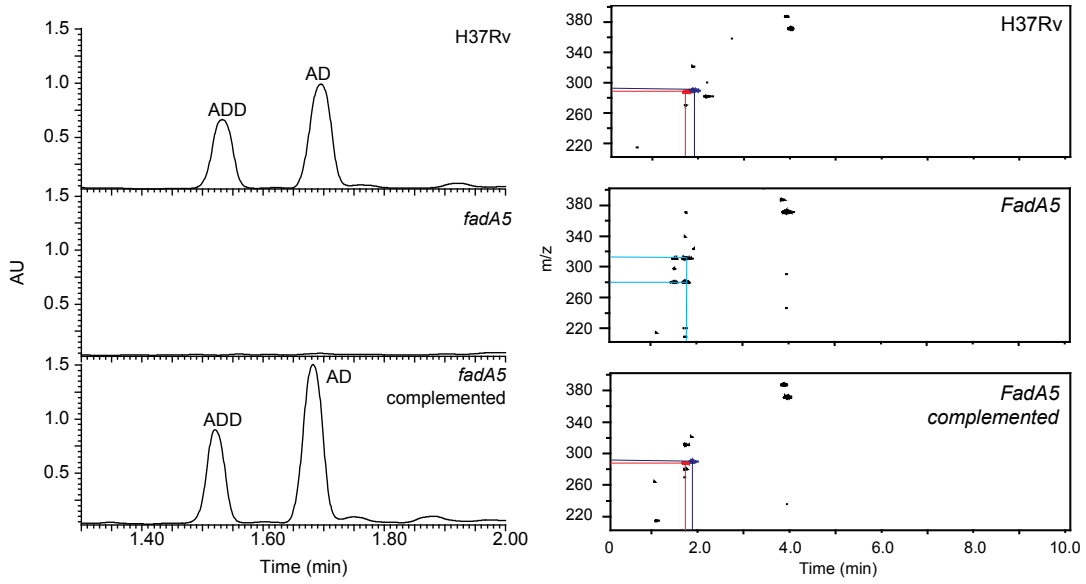


Figure 4-6. FadA5 is required for the production of androst-4-ene-3,17-dione and androsta-1,4-diene-3,17-dione from cholesterol by *M. tb*. (A) Liquid chromatography with detection at 240 nm analysis of H37Rv, *fadA5*, and complemented *fadA5* culture supernatants that were prepared as described in *Materials and Methods.* The chromatographic profile from 1.3 to 2.0 min is shown. (B) LC/MS contour plot of scanning APCi from 200 – 2000 Th in positive ion mode on the Y-axis and time on the X-axis. Only the region from 200 – 400 Th is shown for clarity. There were no ions detected above 400 Th. The cross peaks corresponding to ADD and AD in the H37Rv and complemented *fadA5* samples are delineated with red and dark blue lines, respectively. The cross peak at 1.6 min corresponding to unknown metabolites in the *fadA5* sample is delineated with a light blue line. These profiles are representative of three independent biological replicates. [reprinted with permission from *(1)*; copyright 2009, American Society for Microbiology].

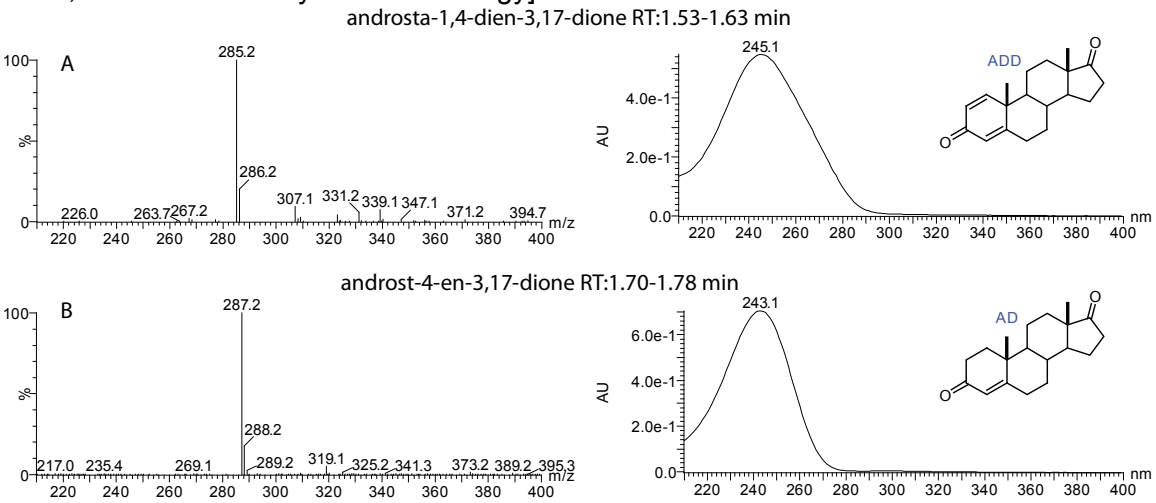


Figure 4-7. LC/MS/UV analysis of peaks observed in Figure 4-6 (H37Rv and *fadA5* complemented) at (A) 1.53 – 1.63 and (B) 1.70 – 1.78 min. The mass spectra were obtained with APCI ionization in positive ion mode. The UV spectra were acquired with a photodiode array detector. [reprinted with permission from *(1)*; copyright 2009, American Society for Microbiology].

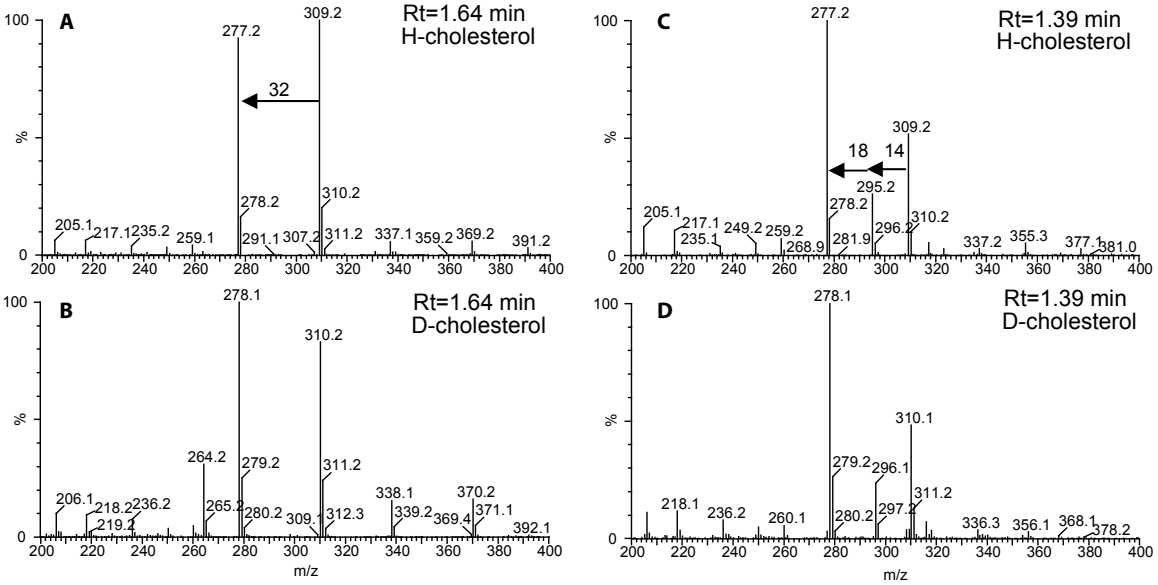
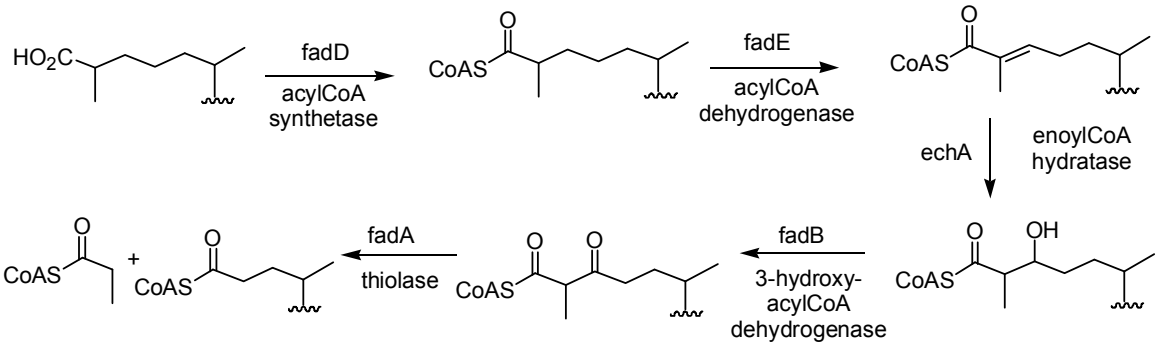


Figure 4-8. LC/MS/UV analysis of peaks observed in Figure 4-6 (*fadA5*) at (A and B) 1.64 and (C and D) 1.39 min. The mass spectra were obtained with APCI ionization in positive ion mode. D-cholesterol is [25,26,26,26,27,27,27-^{2}H]-cholesterol.

Based on the identities of isolated metabolites in other actinomycetes (*19-20*), two β-oxidation cycles have been proposed in the pathway for cholesterol side-chain degradation that ultimately yields the 17-keto androsteroids, e.g., AD and ADD (Scheme 1-2). However, due to the redundant genes encoding β-oxidation in *M. tb*, it is difficult to identify genes specifically responsible for cholesterol chain degradation. *fadA5* was proposed to encode the enzyme for thiolytic cleavage of the β-ketoacyl intermediate(s) in cholesterol side-chain metabolism (Scheme 4-1), and was determined to be required for infection in the late stage in the mice model (*1*). Recombinant *fadA5* was also purified and determined to be a β-ketoacyl CoA thiolase as annotated (*1*). However, direct metabolic products are needed to further identify the gene function *in vivo*. The inhibitors of the enzymes in the ring degradation can be used to block the further metabolism of the ring. Theoretically, the accumulated metabolites will be less complex and of different stages of the chain degradation. The analysis of those metabolites will provide further identification of the gene functions.



Scheme 4-1. One β-oxidation cycle involved in the proposed side-chain degradation of cholesterol in *M. tb*.

Metabolism of cholesterol by the hsaA mutant. *hsaA* is required for *M. tb* growth on cholesterol as an only carbon source. The whole cell metabolism of [4-^{14}C]-cholesterol in wild type and the *hsaA* mutant 24 hours after addition of ^{14}C-cholesterol was analyzed (Figure 4-9A). The *hsaA* mutant metabolizes cholesterol much slower compared to the wild type. First, only less than 10% of the total counts were consumed in the *hsaA* mutant compared to more than half of the ^{14}C mass that is lost in wild type. Second, the *hsaA* mutant accumulated cholesterol and cholest-4-en-3-one: 17% of the original ^{14}C-cholesterol remains in the mutant compared to less than 4% in the wild-type sample. Peaks between 0 and 2 min accumulated in the *hsaA* mutant (~27% of total counts), and absorb UV between 230 and 240 nm, which is consistent with the formation of more hydrophilic A-ring oxidized products expected from accumulating metabolites due to disruption of the *hsaA* gene (Scheme 1-2) and the partial degradation of the C17 side chain. On the other hand, the *hsaA* mutant accumulates several more hydrophobic metabolites with retention time between 7 and 10 minutes, accounting for 50% of the ^{14}C mass as compared to 25% in wild type. We hypothesize that blocking of further ring degradation results in more flux into the lipid metabolites as a storage form of cholesterol or other functional lipids.

Metabolism of cholesterol by the fadE29 mutant. The whole cell metabolism of [4-^{14}C]-cholesterol in wild type and the *fadE29* mutant 5 hours after addition of ^{14}C-

cholesterol was analyzed (Figure 4-9B). This mutation is not in frame and most likely results in disruption of at least part of the igr operon. *fadE29 (Rv3543c)* is required for growth on cholesterol as a sole carbon source and its mutation causes attenuation of virulence in mouse infection (Dubnau, pers. comm.). The *fadE29* mutant accumulated cholesterol and cholest-4-en-3-one: 23% of the original ^{14}C-cholesterol remains in the mutant compared to ~5% in the wild-type sample. After 5 hours, there are mainly two peaks accumulated in the *fadE29* mutant: one is the peak with a retention time of 1.9 min with no UV absorption spectrum and another one with a retention time around 6.7 -7 min which was detected in CDC1551 and H37Rv wild type at 2 hours and 24 hours. Accumulation of a proposed storage waxy ester as well as other specific hydrophobic lipids is consistent with the reduced metabolism of cholesterol in the mutant.

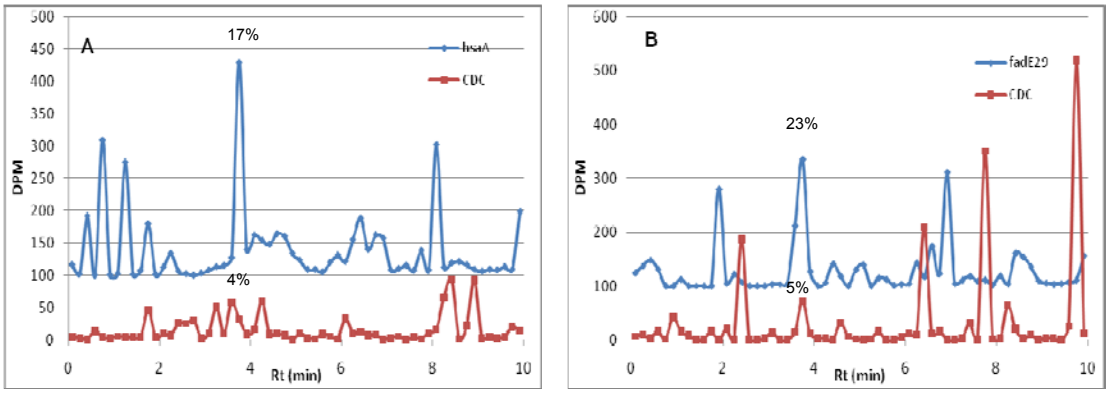


Figure 4-9. LC/UV analysis of (A) wild-type CDC1551 and *hsaA* mutant with [4-^{14}C]-cholesterol for 24 h, and (B) wild-type CDC and *fadE29* mutant with [4-^{14}C]-cholesterol for 5 h. Chloroform: methanol extracts of *M. tb* cells were analyzed by LC, and were collected every 10 sec, and counts per fraction were determined by liquid scintillation counting. The peak eluting at 3.7 min contains cholesterol and cholest-4-en-3-one. Data shown are representative of three independent experiments.

Identification of a cholesterol-derived apolar lipid in wild-types and mutants. A cholesterol-derived apolar lipid was observed in the cells of wild type, and the *hsd, fadA5, hsaA* and *fadE29* mutants (Table 4-2). The implications of this experiment are as follows. First, the metabolism of the cholesterol-derived apolar lipid is much faster in

CDC1551 than in H37Rv. The amount of the cholesterol-derived apolar lipid decreases in CDC1551 from 5 h to 24 h, however the amount of this lipid increases in H37Rv from 24 h to 48 h (data not shown). This dynamic trend fits the metabolism of cholesterol as discussed before, which supports the idea that this lipid is formed as a storage form of cholesterol, which may be converted back to cholesterol after the free cholesterol is consumed or directly degraded. Second, the formation of this lipid in the *hsd* mutant is slower than that in the wild type which means that the lack of 3β-hydroxyl oxidation activity slows down the formation of this lipid. The same phenomenon was observed when cholesteryl chloride was incubated with H37Rv cells: the formation of this lipid is at least 20 fold slower compared to that in *M. tb* H37Rv incubated with cholesterol. However, since the formation is not completely blocked, there might be alternative pathways that function similarly but slower. Third, more of the cholesterol-derived apolar lipid was formed in the *fadE29* and *fadA5* mutants compared to their respective wild types. One possible explanation is that the cholesterol-derived apolar lipid accumulates when the cholesterol degradation pathway is hindered. We hypothesized that this lipid and the peaks around that range represent a distinct metabolic or storage pathway of cholesterol.

Table 4-2. Relative amount of cholesterol-derived apolar lipid compared to the total extractable lipids from cells based on the ^{14}C counts.

Strains	H37Rv	*fadA5*	CDC1551	CDC1551	*hsd*	*fadE29*	*hsaA*
% formation	43	51	3	14	2	23	7
time	24	24	24	5	5	5	24

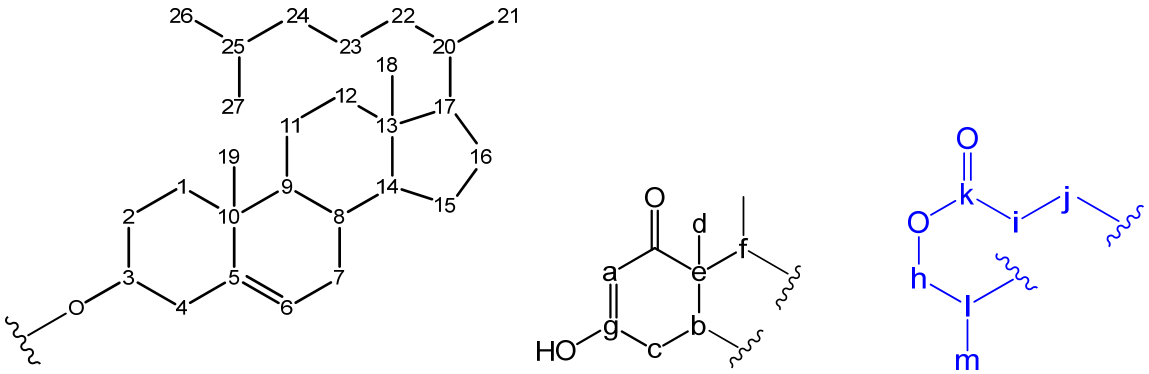
This compound shows a λ_{max} of 241 nm and IR absorbance at 1736 cm^{-1} (an unsaturated ester or a lactone) and 1675 cm^{-1} (an α, β-unsaturated carbonyl group, Figure 4-11). In the Orbitrap MS^2 mass spectrum, this compound has an MNa^+ of 891.6808 that fragments to 523.3391 ($C_{31}H_{48}O_5Na^+$, calculated MNa^+: 523.33995) with loss of 368.3417 (cholesteryl, calculated M: 368.3443), and the fragment produces no

further fragments. MS analysis of this compound from cells incubated with cholesterol deuterated only in the side chain (cholesterol-25,26,26,26,27,27,27-d_7) and cholesterol deuterated only in A/B rings (cholesterol-2,2,3,4,4,6-d_6) shows the mass increase of 13 Th and 10 Th, respectively, compared to the non-labeled sample, indicating that the compound contains two cholesterol-derived units. One unit is confirmed to be cholesteryl, and the ^{1}H, ^{13}C, and ^{1}H-^{13}C HMBC NMR data of the cholesterol unit are reported in Table 4-3. Since the cholesteryl is unmodified in structure, it is quite possible that the other cholesterol-derived unit has a loss of one deuterium in the tail and 2 deuteria in the A/B rings.

The other part of the structure is partially determined due to the overlap of signals. Two sets of methyl groups (0.71, 12.18 ppm; 1.18, 17.60 ppm) have similar correlation to carbons compared to C18 and C19 in cholesteryl motif, suggesting the similar structural environments of those methyl groups (Figure 4-12B). There are two correlation systems are indicated in Figure 4-12A, in black and blue, respectively. The HMQC spectrum shows a sp^2 carbon at 5.72 and 123.98 ppm (a), which is correlated to the carbons at 33.16 ppm (b) and 38.81 ppm (c) in the HMBC spectrum. The carbon at 38.81 ppm is correlated to a methyl group (1.18, 17.60 ppm) (d), which is also correlated to carbons at 35.36 (e), 53.96 (f), and 171.76 ppm (g). The carbon at 171.76 ppm is further correlated to a proton at 2.38 ppm (c), which is also correlated to carbons at 32.35 and 123.98 ppm (a) (Figure 4-12A, 4-10B). Another correlation system suggests the correlation of the following carbons: h (3.98/3.86, 69.99 ppm, CH_2), i (2.60, 29.51 ppm, CH_2), j (2.62, 29.77 ppm, CH_2), k (172.66 ppm, C), l (1.76, 32.13 ppm, CH), and m (0.92, 17.20 ppm, CH_3) (Figure 4-12A, 4-10C). The chemical cleavage of the compound and long-range COSY spectrum will help determine the structure and the final confirmation of this structure will require X-ray crystal analysis.

Table 4-3. Chemical shifts of the cholesterol motif. (spectra taken in $CDCl_3$, 300K)

Atom #	Multiplicity	δH (ppm)	δC (ppm)	HMBC
1	CH_2	1.12/1.33	24.06	H2,H19
2	CH_2	1.58/1.85	27.97	H4
3	CH	4.62	74.55	H2,H4
4	CH_2	2.32	38.30	H6
5	C	-	139.80	H2,H4,H7,H19
6	CH	5.37	122.91	H4,H7
7	CH_2	1.53/1.96	32.27	H6
8	CH	1.50	21.25	-
9	CH	0.93	50.25	H8, H12,H19
10	C	-	36.81	H4,H6,H11,H19
11	CH_2	1.44	32.08	H12, H9
12	CH_2	1.17/2.0	39.96	H18
13	C	-	42.62	H12,H14,H17,H18
14	CH	1.02	56.38	H18
15	CH_2	1.07/1.55	24.50	H14
16	CH_2	1.26/1.82	28.23	H15, H17
17	CH	1.09	56.28	H12,H18,H21, H23
18	CH_3	0.68	12.08	H12, H14, H17
19	CH_3	1.03	19.53	H1, H9
20	CH	1.36	36.01	H17, H21
21	CH_3	0.91	18.94	H17, H20
22	CH_2	0.99/1.35	36.01	H21
23	CH_2	1.15	39.85	H21
24	CH_2	1.41/2.01	37.19	H26/27
25	CH	1.51	27.97	H26/27
26	CH_3	0.86	23.03	H25,H27
27	CH_3	0.86	22.77	H25,H26



A B C

Figure 4-10. Structure of the cholesterol motif (A) and two predicted correlation systems (B and C).

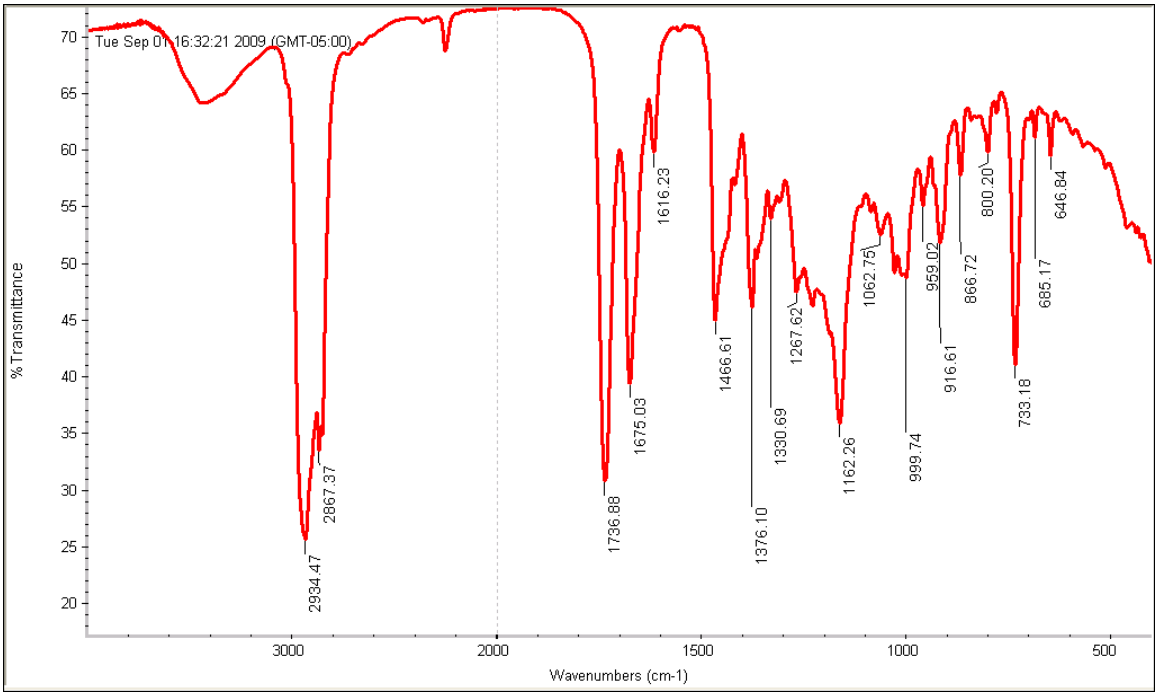


Figure 4-11. IR spectrum of the cholesterol-derived apolar lipid.

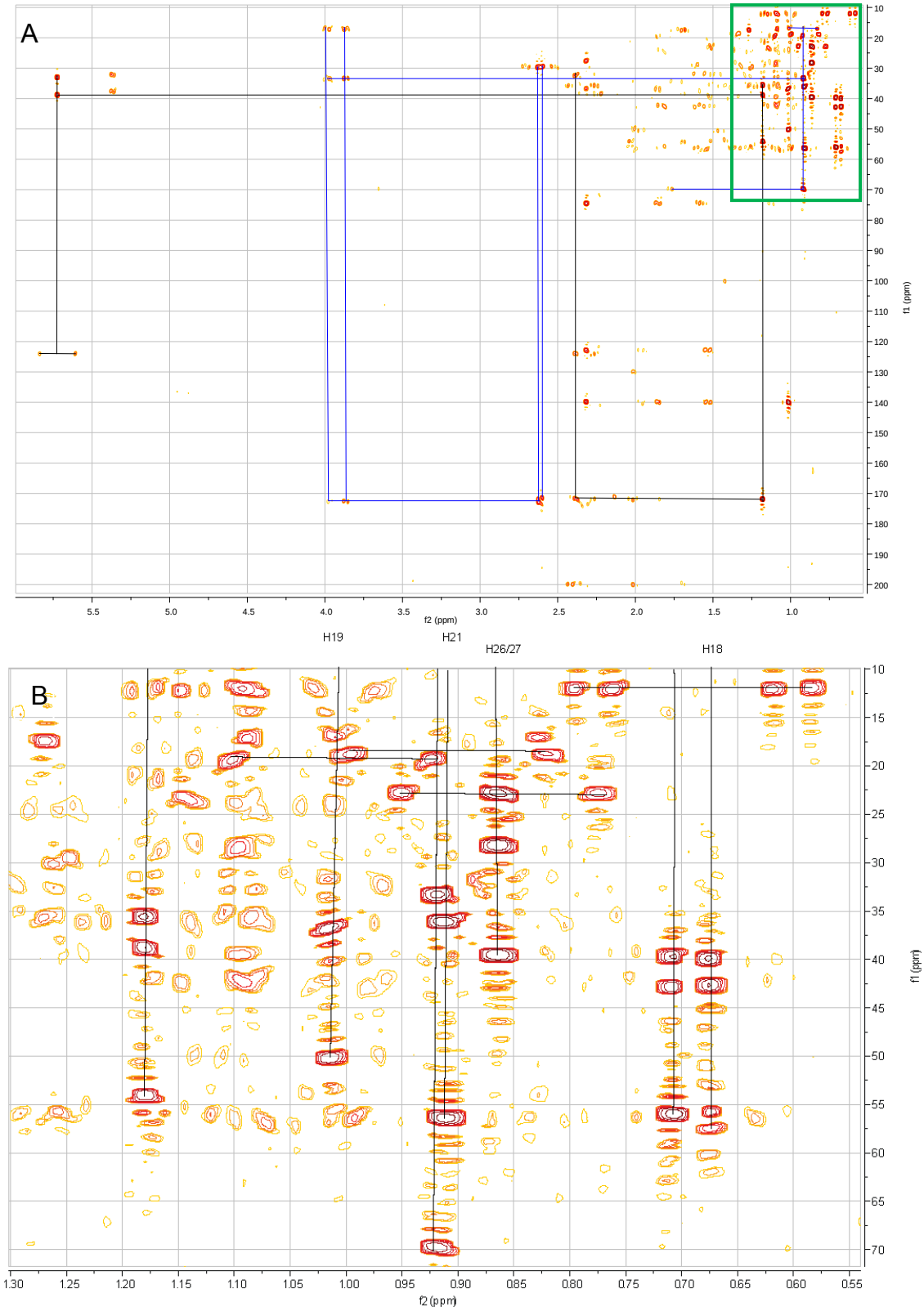


Figure 4-12. ^{1}H-^{13}C HMBC spectrum (A) and the magnified display (B) of the region boxed in a rectangle in (A) of the cholesterol-derived apolar lipid.

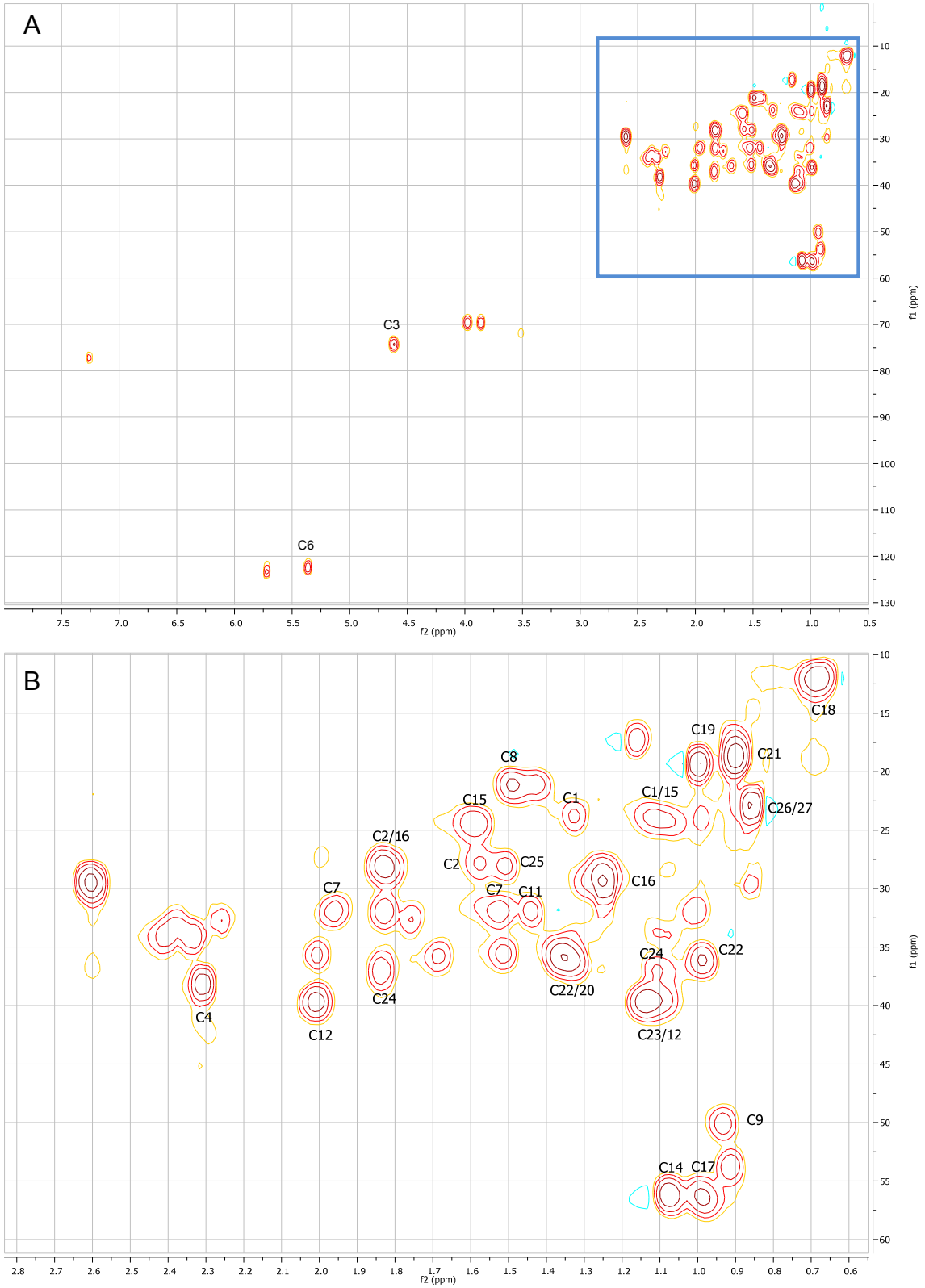


Figure 4-13. ^{1}H-^{13}C HSQC spectrum (A) and the magnified display (B) of the region boxed in a rectangle in (A) of the cholesterol-derived apolar lipid.

Overall, we investigated the function of several genes in the cholesterol metabolic pathway in *M. tb* and present evidence that Hsd is responsible for 3β-hydroxysteroid oxidation, the first step in the ring degradation, and FadA5 may be involved in the side-chain degradation. Both *hsd* and *fadA5* are required for the utilization of cholesterol as a sole carbon source. In addition, *fadA5* is required for the virulence of *M. tb* in the chronic stage of mouse lung infection. We show that the *fadA5* mutant strain is defective for the activity of metabolizing cholesterol to androst-4-ene-3,17-dione and androsta-1,4-diene-3,17-dione and exporting these metabolites into the medium. The accumulation of these metabolites or the lack of further metabolites may be related to the *M. tb* virulence. Further investigation of the genes and enzymes involved in this pathway will help understand the metabolism of cholesterol and its relationship to *M. tb* virulence.

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Chapter 5

Analysis of Extractable Lipids in *M. tuberculosis*

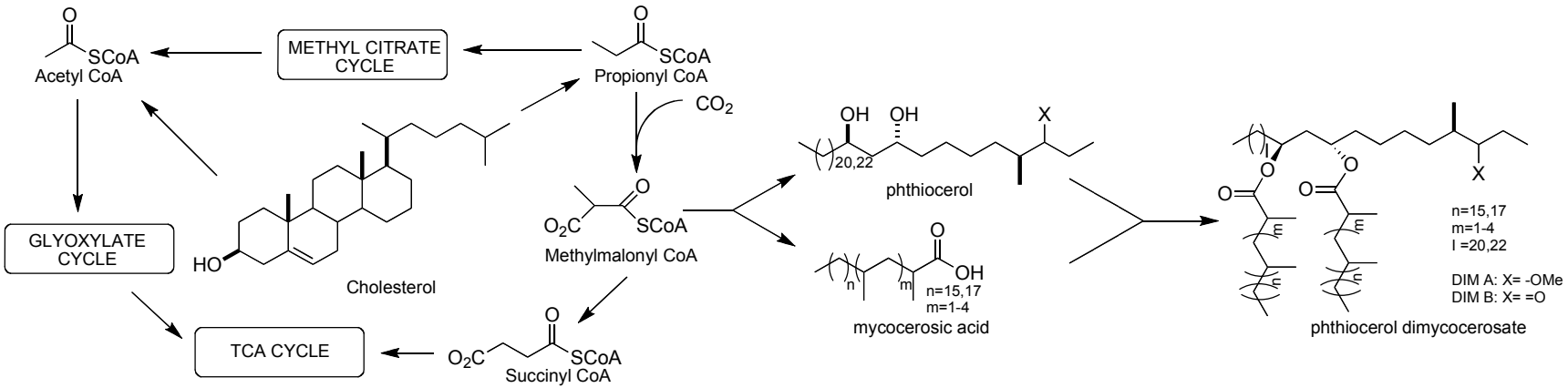
This chapter is adapted from a paper that was published in *Biochemistry (1).*

I. Introduction

M. tb adapts to the intracellular host environment by shifting to a lipid metabolism in the glucose-deficient milieu of the macrophage (*2-3*). Catabolism of lipids via β-oxidation in conjunction with the anaplerotic glyoxalate cycle supports energy production. However, β-oxidation of branched and odd-chain fatty acids and lipids like cholesterol yields propionyl CoA in addition to acetyl CoA (*4-8*). (Propionate is also produced from degradation of branched amino acids (*9*).) The accumulation of propionate is toxic and it is shunted into the methyl citrate cycle and the methylmalonyl pathway. In addition to anapleuorosis of succinate, the methylmalonyl pathway provides methylmalonyl CoA for the biosynthesis of methyl-branched fatty acids. These methyl branched fatty acids are in turn incorporated into complex bacterial lipids (Scheme 5-1).

Cholesterol is abundant in the intracellular environment and survival of *M. tb* in the host requires the Mce4 lipid transporter which can function to take up cholesterol (*10*). Furthermore, metabolic labeling studies with *M. tb*, demonstrated that cholesterol degradation products can be converted to both CO_2 and to phthiocerol dimycoserate (PDIM) (*10*). The CO_2 is generated during ATP formation in the TCA cycle. The label in PDIM is thought to derive from propionyl CoA that is converted to methylmalonyl CoA in a reaction catalyzed by pyruvate carboxylase.

Although not essential for viability, several lipids found on the surface of the bacterial cell are proposed to play a role in down-regulation of the host response (*11*). In addition to PDIM, the lipids include acyltrehaloses, sulfolipids, liparabinomannan, diacyl and polyacyltrehaloses, di and tri-mycolates, and phenolic glycolipids. The lipids contain methyl-branched long chain fatty acids, like mycocerosic, mycolipenic and mycolipanoic acids. These fatty acids are synthesized by individual fatty acid synthase complexes from malonyl and methylmalonyl CoA and their biosynthesis requires a



Scheme 5-1. Flux of metabolites from cholesterol catabolism. [reprinted with permission from (*1)*; copyright 2009, American Chemical Society].

pool of acetyl and propionyl CoA.

The average mass of PDIM increases ~52 a.m.u. when the in vitro culture propionate concentration is increased from 0 to 1000 μM (*5*). That is, the chain length of the methyl branched mycocerosic acid increases with increasing concentrations of propionate. Growth on valerate, the β-oxidation of which yields one acetate and one propionate, elicits a similar mass shift. Growth on butyrate, the β-oxidation of which yields two acetates does not result in an increase in PDIM molecular weight. The higher mass PDIMs observed upon growth on propionate *in vitro* are also observed in bacteria infecting the mouse lung (*2, 5*). Cholesterol may serve as a source of propionate *in vivo* and we asked whether catabolism of cholesterol results in a sufficient intracellular pool of propionate to increase methyl-branched fatty acid biosynthesis by *M. tb.* Specifically, we investigated whether sufficient propionyl CoA can be derived from *M. tb* growth on cholesterol as a sole carbon source or as an additive to standard medium to increase cellular flux into the methylmalonyl CoA pathway with a consequent alteration of PDIM molecular weight.

The degradation pathway of cholesterol in *M. tb* is partially elucidated, although genes have not been definitively assigned to all steps (*12-15*). β-oxidation of the side chain yields at least one propionyl CoA and one acetyl CoA per molecule of cholesterol. Non-enzymatic cleavage of the C22 carboxylic acid to yield testosterone and propionyl CoA has been proposed (*16*). However, it is unlikely that there is an uncatalyzed step in catabolism and α-oxidation may occur. Ring cleavage can yield at least one more propionyl CoA molecule per molecule of cholesterol. The exact ratio of acetate and propionate obtained from cholesterol degradation depends on the identity of the ultimate products that have not been fully identified in *M. tb*.

The mass of isolated PDIM was used as a reporter for intracellular propionate metabolic flux. In addition to PDIM, the apolar lipids profiles of wild-type and selected

mutant strains were analyzed to help elucidate the function of the genes.

II. Experimental Methods

Bacterial strains, media and growth conditions. M. tuberculosis cultures were grown at 37 °C in Middlebrook 7H9 liquid media (Becton Dickinson), supplemented with 0.05% Tween-80, 10% albumin-dextrose-NaCl complex (ADN) (*17*) and 0.2% glycerol, or on Middlebrook 7H10 plates supplemented the same way (referred to as standard medium). Kanamycin was added at 20 $g \cdot ml^{-1}$ and hygromycin at 100 $\mu g \cdot ml^{-1}$. Growth on cholesterol as a sole carbon source was done by supplementing 7H9 media with 1 $mg \cdot ml^{-1}$ cholesterol made up in the nonionic surfactant tyloxapol (Sigma). The stock solution was 20 $mg \cdot ml^{-1}$ cholesterol made in pure tyloxapol, autoclaved, and then boiled to dissolve the cholesterol completely. When this is diluted 20-fold into 7H9, the cholesterol does not precipitate and the growth of the culture can be followed by measuring optical density (OD) at 540 nm. When cholesterol was used as an additive to standard medium, cholesterol (1 $mg \cdot ml^{-1}$ final concentration) in Tween-80 (1% w/v final concentration) was added to the standard medium described above. Cholesterol stock solutions (20 $mg \cdot ml^{-1}$) were prepared in aqueous Tween-80 (20% w/v). The wild-type *M.tb* H37Rv and CDC1551, and the mutant strains, including *fadA5*, *fadA5* complement, *hsd*, *hsd* complement, *fadE29*, *hsaA*, *fadD19* and *mmpL5* (cloning and construction of those strains is described in Chapter 4) were used.

Mass spectrometric analysis of apolar lipids. Apolar lipids were extracted twice using petroleum ether as described (*18*). Cells pellets from 40 mL of culture were resuspended in 20 mL of methanol:0.3% aqueous sodium chloride (10:1). 10 mL of petroleum ether was added to the cells, stirring for 15 min, and the upper layer was removed. Another 10 mL of petroleum ether was added to the lower aqueous phase

and stirred for 15 min. The upper layer was removed and combined with the previously removed upper layer. Those combined layers contain apolar lipids. Apolar lipids were purified by solid phase extraction using silica gel. After elution with petroleum ether followed by petroleum ether:ethyl acetate (100:1) fractions containing PDIM were dried under nitrogen gas and resuspended in chloroform:methanol (2:1). Total lipids were extracted with chloroform and methanol using the method of Bligh and Dyer (as described in Chapter 4) (*19*). Mass spectrometry was performed on an LTQ-Orbitrap (Thermo Scientific) equipped with an electrospray source operating in positive ion mode with an ionization voltage of 5 kV, capillary voltage of 34 V and tube lens of 150 V. Collision induced dissociation (CID) was applied to the PDIM ions to generate structural data.

III. Result and discussion

Cholesterol is a significant source of propionate in the absence of sugar carbon sources. We prepared apolar lipid extracts for mass spectral analysis of PDIM from wild-type H37Rv *M. tb* grown on standard medium (7H9 salts supplemented with glycerol, albumin, dextrose, and catalase), grown on standard medium supplemented with cholesterol, and grown on 7H9 salts supplemented with only cholesterol. The extracts were analyzed by high-resolution mass spectrometry using electrospray ionization on an LTQ Orbitrap. PDIM was a mixture of DIM A (methoxy ether) and DIM B (ketone) in the wild-type *M. tb* grown in the standard medium (Figure 5-1). DIM A is 16 mass units higher than DIM B with the same lipid and polyketide chains. Both contain mycocerosic acid, a methyl-branched long chain fatty acid that is esterified to phthiocerol or phenylphthiocerol. Characteristic of fatty acids, a family of peaks separated by 14 mass units is observed at 1318, 1332, 1346, 1360, 1374, 1388, 1402, 1416, 1430, 1444, 1458,

and 1472 for DIM B. A similar series for DIM A is observed at 1334, 1362, 1376, 1390, 1404, 1418, 1432, 1446, 1460, 1474 and 1488. The molecular weight distribution and DIM A/B content was unchanged in wild-type *M. tb* grown on standard medium that is supplemented with 2.6 mM cholesterol (Figure 5-1B). In contrast, when cholesterol is the sole carbon source, only DIM B is observed and its average molecular weight is shifted 42 a.m.u. higher (Figure 5-1C). This increase in mass was due to the incorporation of longer mycoserosic acids into PDIM as determined by MS^2 fragmentation (Figure 5-2). These data support the hypothesis that propionyl CoA is formed during the catabolism of cholesterol and that the intracellular propionate concentration is elevated sufficiently to increase the metabolic flux into methylmalonyl CoA biosynthesis. The MWs observed are similar to those seen upon growth on 500-1000 μM propionate (*5*). However, the presence of cholesterol is insufficient to alter the metabolic flux when alternative sugar-based carbons sources, e.g., glycerol or dextrose, are present.

Just as was observed upon growth on 1000 μM propionate and in infected mouse lungs (*5*), when wild-type *M. tb* was grown using cholesterol as the sole carbon source only DIM B is detected (Figure 5-3). This abundance of the ketone form of PDIM suggests that the cellular pool of NADH is reduced upon cholesterol catabolism. Alternatively, the ketoreductase (*Rv2951c*) that catalyzes the conversion of the ketone DIM B form to the alcohol precursor of DIM A (*20*) may be inhibited or down-regulated. However, the loss of DIM A does not affect virulence (*20*).

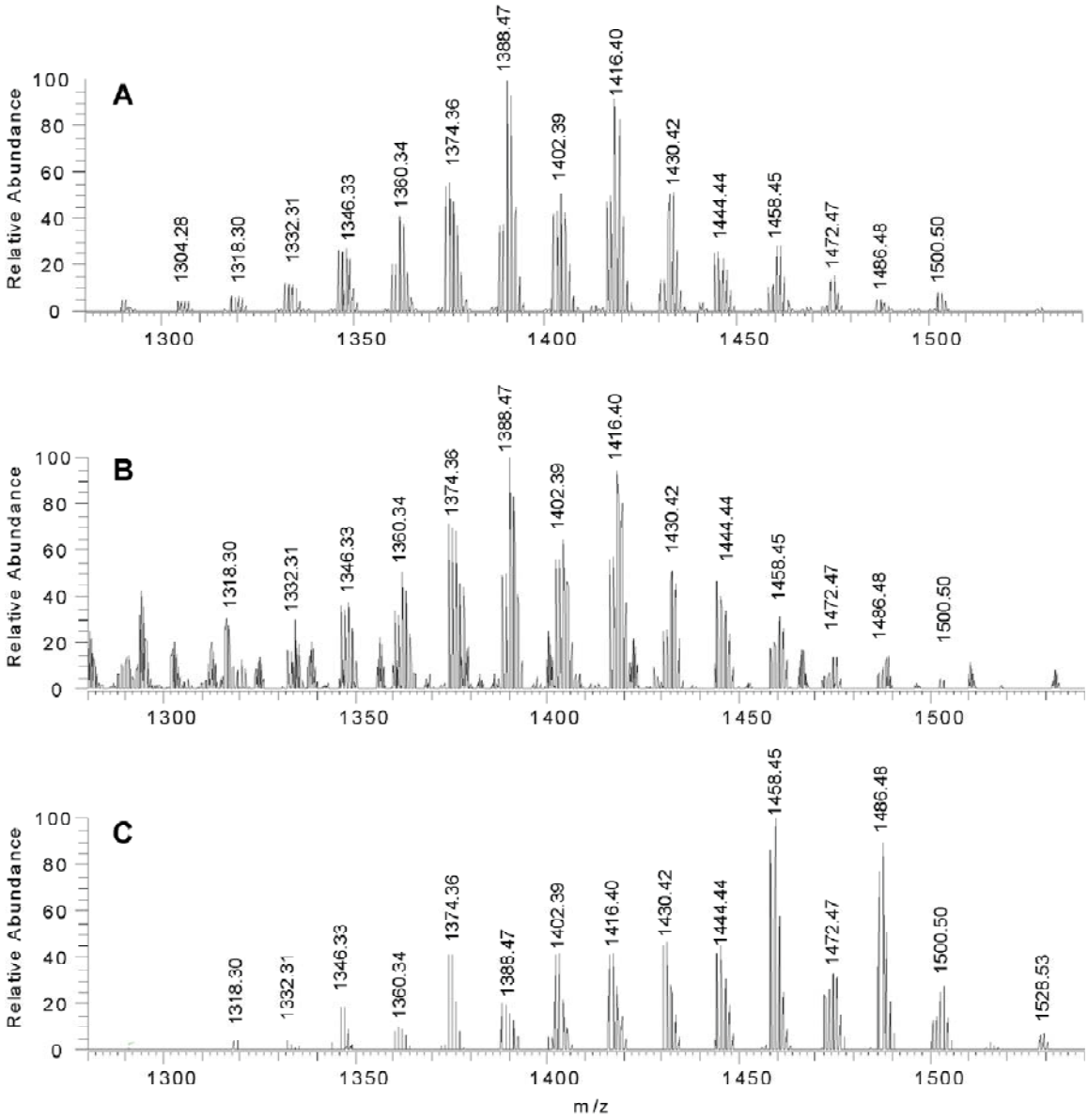


Figure 5-1. Mass spectrometric analysis of apolar lipids of wild-type H37Rv *M. tb*. (A) *M. tb* grown in standard media，(B) *M. tb* grown in standard medium supplemented with 2.6 mM cholesterol solubilized in Tween-80 (1% w/v) and (C) *M. tb* grown using 2.6 mM cholesterol as the sole carbon source solubilized with tyloxapol (5% w/v). The additional peaks that appear in spectrum B are due to doubly charged detergent (Tween-80) ions. [adapted with permission from (*1)*; copyright 2009, American Chemical Society].

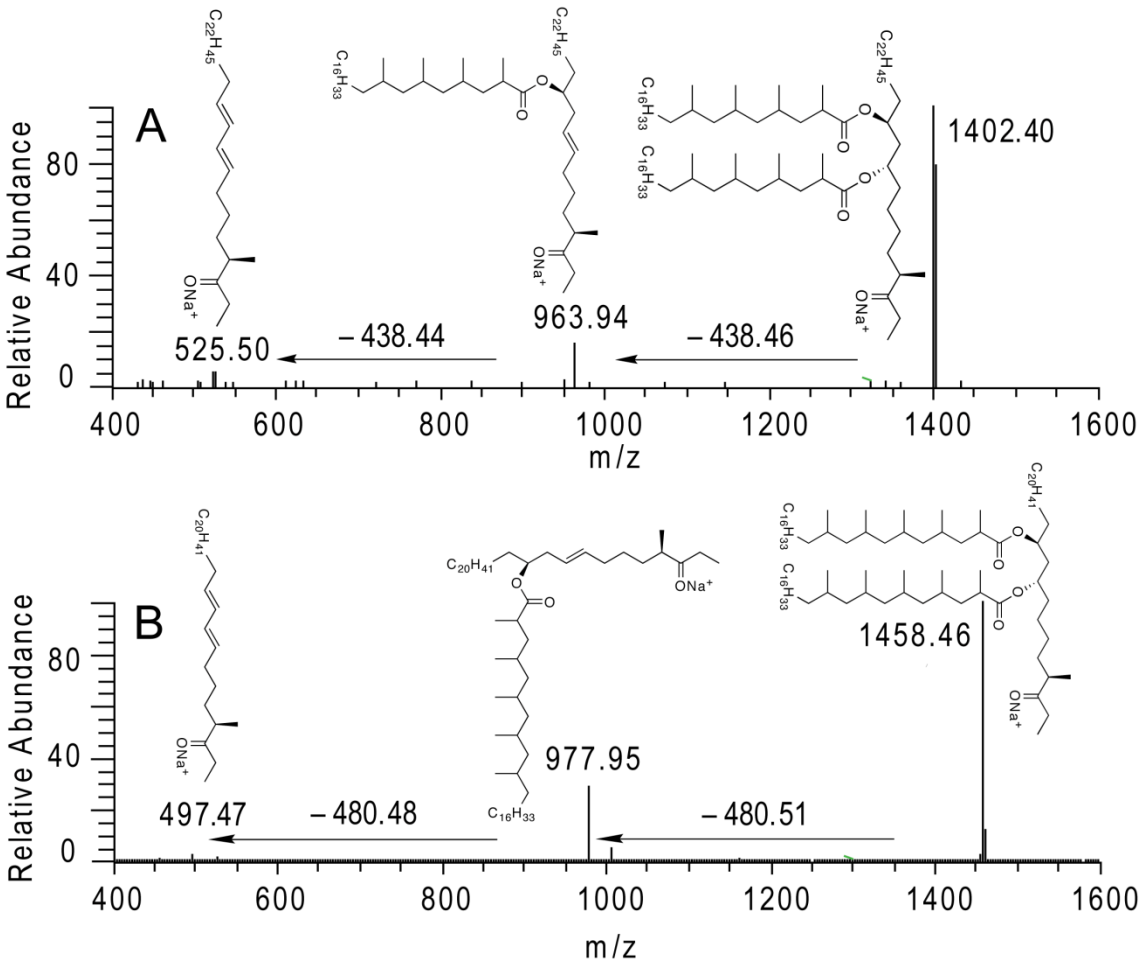


Figure 5-2. Mass spectrometric fragmentation analysis of apolar lipids of wild-type H37Rv *M. tb*. (A) *M. tb* grown in standard media. The parent ion at 1402.4 in Figure 5-1A was selected for fragmentation. (B) *M. tb* grown using cholesterol as the sole carbon source. The parent ion at 1458.46 in Figure 5-1C was selected for fragmentation. [reprinted with permission from (*1)*; copyright 2009, American Chemical Society].

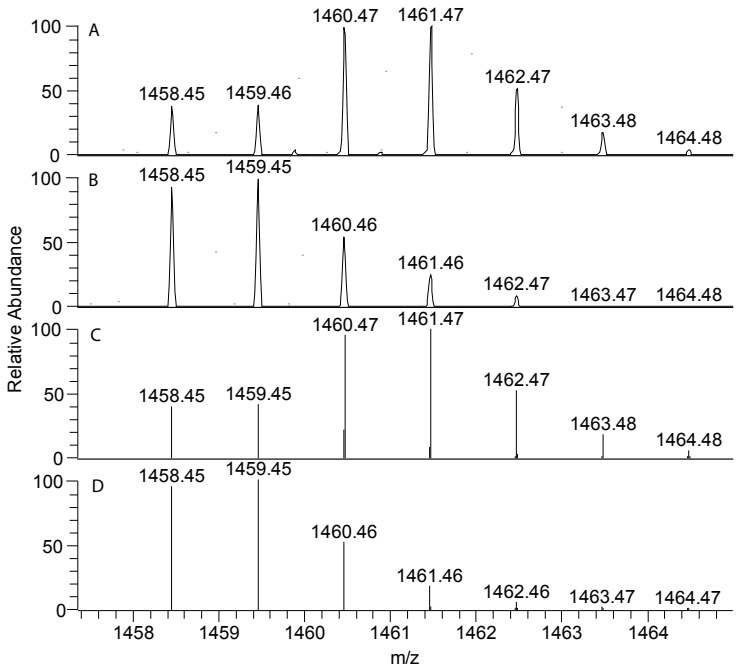


Figure 5-3. A magnified view of a single isotope packet of apolar lipids of wild-type H37Rv *M. tb*. *(A) M. tb* grown in standard media. *(B) M. tb* grown using 2.6 mM cholesterol as the sole carbon source solubilized with tyloxapol (5% w/v) *(C)* Natural isotope distribution of 71% $C_{97}H_{192}NaO_5$ (DIM A) and 29% $C_{97}H_{190}NaO_5$ (DIM B). *(D)* Natural isotope distribution of 100% $C_{97}H_{190}NaO_5$ (DIM B).

In the *hsaA* mutant, both truncated PDIM (Figure 5-4A) and PDIM (Figure 5-4B) were observed with clear distribution of peaks separated by 14 mass units. The distribution of PDIM is different from that in wild type, indicating the mutation of *hsaA* may affect the uptake of other carbon source by *M. tb*. The molecular weight distribution shows two sets of dominant peaks in PDIM: 1374 and 1458. However, only DIM B is shown in *hsaA* mutant for non-clarified reasons. DIM B at 1374 in *hsaA* mutant shares the same lipid and polyketide chain of DIM A at 1390 in wild-type *H37Rv* grown in the standard media (Figure 5-1A), which is also the highest peak. And the DIM B at 1458 is the same as the highest peak in wild-type H37Rv grown using cholesterol as the sole carbon source (Figure 5-1C). The mass difference between 1374 and 1458 is 84.09 (2×-$CH(CH_3)CH_2$-), corresponding to the incorporation of two propionate units. This is also observed in truncated PDIM in the *hsaA* mutant. The truncated PDIM was

observed in both wild types and the mutant strains, although varying in the relative amount compared to PDIM. The truncated PDIM contains the phthiocerol chain and only one mycocerosic chain (*21*). Two sets of dominant peaks in truncated PDIM are 914 and 956 (shown as a dominant peak of truncated PDIM in wild-type *H37Rv* grown using cholesterol as the sole carbon source), with the mass difference of 42.0466 ($-CH(CH_3)CH_2-$). The results confirm that one propionate unit produced from cholesterol is added to each mycocerosic chain by the polyketide synthesis machinery.

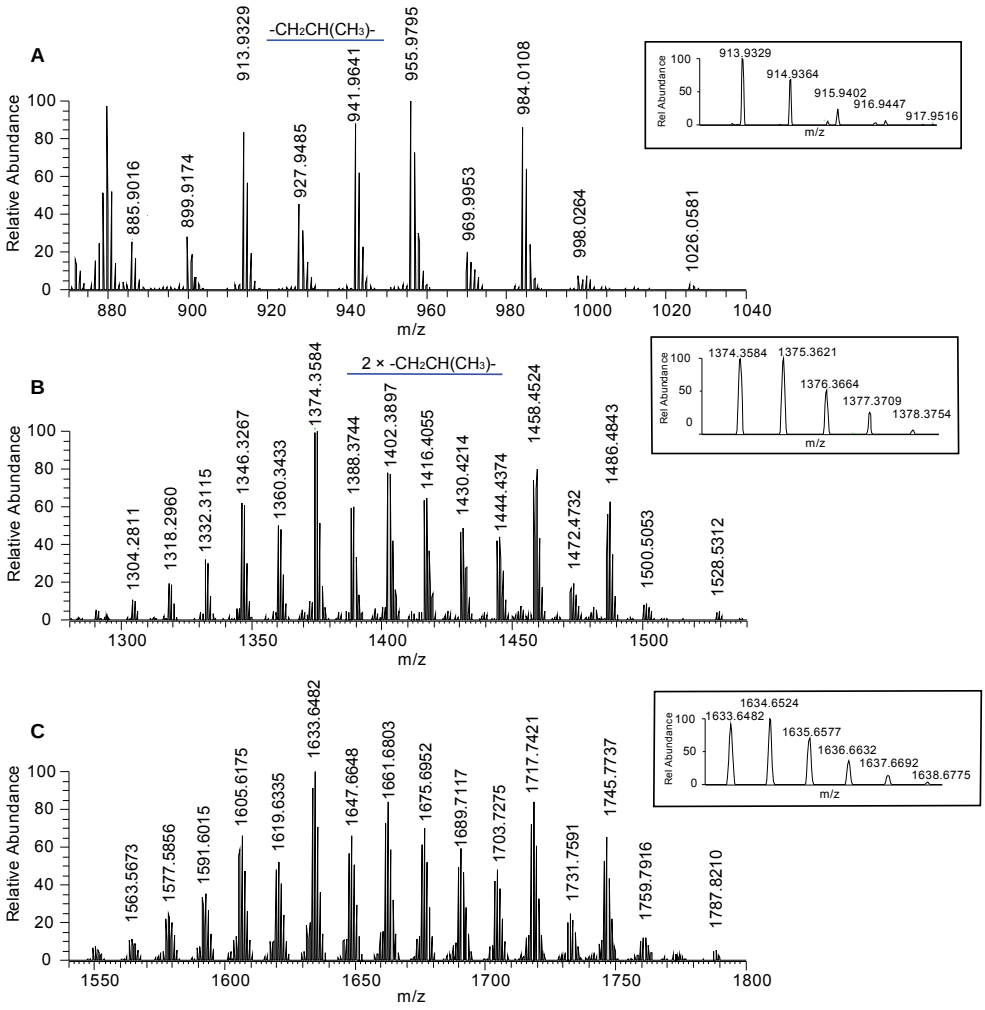


Figure 5-4. Lipids from the *hsaA* mutant grown in standard medium supplemented with 2.6 mM cholesterol solubilized in Tween-80 (1% w/v). (A) Truncated PDIM. Expected mass: 913.9310 ($C_{62}H_{121}O_3^+$) ±14n. (B) PDIM. Expected mass: 1374.3566 ($C_{91}H_{178}NaO_5^+$) ±14n. (C) L_{1633}. Expected mass: 1633.6466 ($C_{109}H_{214}O_6N^+$) ±14n. A magnified view of a single isotope packet is shown in the upper right hand corner of each graph.

A modified PDIM is formed by wild type and the hsaA, hsd, fadD19, and mmpL5 mutants during growth on cholesterol, but not in fadE29. A family of peaks separated by 14 mass units is observed at 1563, 1577, 1591, 1605, 1619, 1633, 1647, 1661, 1675, 1689, 1703, 1717, 1731, 1745 and 1759 (referred as L_{1633}) in wild type, the hsaA, hsd, fadD19 and mmpL5 mutants in standard medium supplemented with cholesterol (Figure 5-5), but not in fadE29. fadA5 does not biosynthesize PDIM due to a spontaneous mutation (22). The hsaA mutant was used as a model to characterize the relationship between PDIM and L_{1633} (Figure 5-4C). The molecular weight distribution of PDIM and L_{1633} is the same, suggesting a direct correlation between these two lipids. Taking the corresponding peaks, e.g. PDIM at 1374.3584 and L_{1633} at 1633.6482, the mass difference is calculated to be 259.2894, which was also observed in other strains. PDIM is ionized as a Na^+ adduct, then the modified molecule would be $C_{18}H_{35}NO$ (calculated mass: 259.2899+Na^+-H^+) if L_{1633} is a H^+ adduct, or $C_{16}H_{37}NO$ (calculated mass: 259.2870) if L_{1633} is a Na^+ adduct. The latter molecular formula is not structurally possible. Therefore, PDIM is modified by $C_{18}H_{35}NO$. However L_{1633} is also missing in the wild-type H37Rv grown with cholesterol as a sole carbon source, which indicates the formation of L_{1633} may be due to the formation of an artifact in the presence of Tween-80. Tween-80 is a C18:1 fatty acid ester of polyoxyethylene and a possible source of oleic acid or $C_{18}H_{35}NO$ with the formation of an amide.

Other apolar lipids are shown in Figure 5-5 B and C. There is a series of lipids with molecular weights around 1942 in the *hsd* mutant and the signals of peaks in the sample treated with the tail-labeled D-cholesterol showed 3 mass units higher compared to those with ring-labeled d-cholesterol, indicating cholesterol is involved in the lipid biosynthesis. Another series of lipids with molecular weights around 2232 was observed in the *hsaA* mutant with unknown structure.

In conclusion, we have used high-resolution mass spectral analysis of a complex

lipid as a reporter for the intracellular formation of propionate upon sterol catabolism. The mass shifts in the PDIM molecular weight distribution observed upon growth on cholesterol as a sole carbon source are analogous to those seen upon growth on 500-1000 μM propionate. Importantly, this same mass shift is not observed upon supplementation of sugar carbon sources with cholesterol. Thus, the metabolic pool of propionate is only increased upon limiting the availability of acetate-forming carbon sources. This observation and the previously observed higher molecular weight PDIMs in mouse lungs (*5*) suggest that in the host, methyl-branched lipid carbon sources are the primary source of nutrition for *M. tb*.

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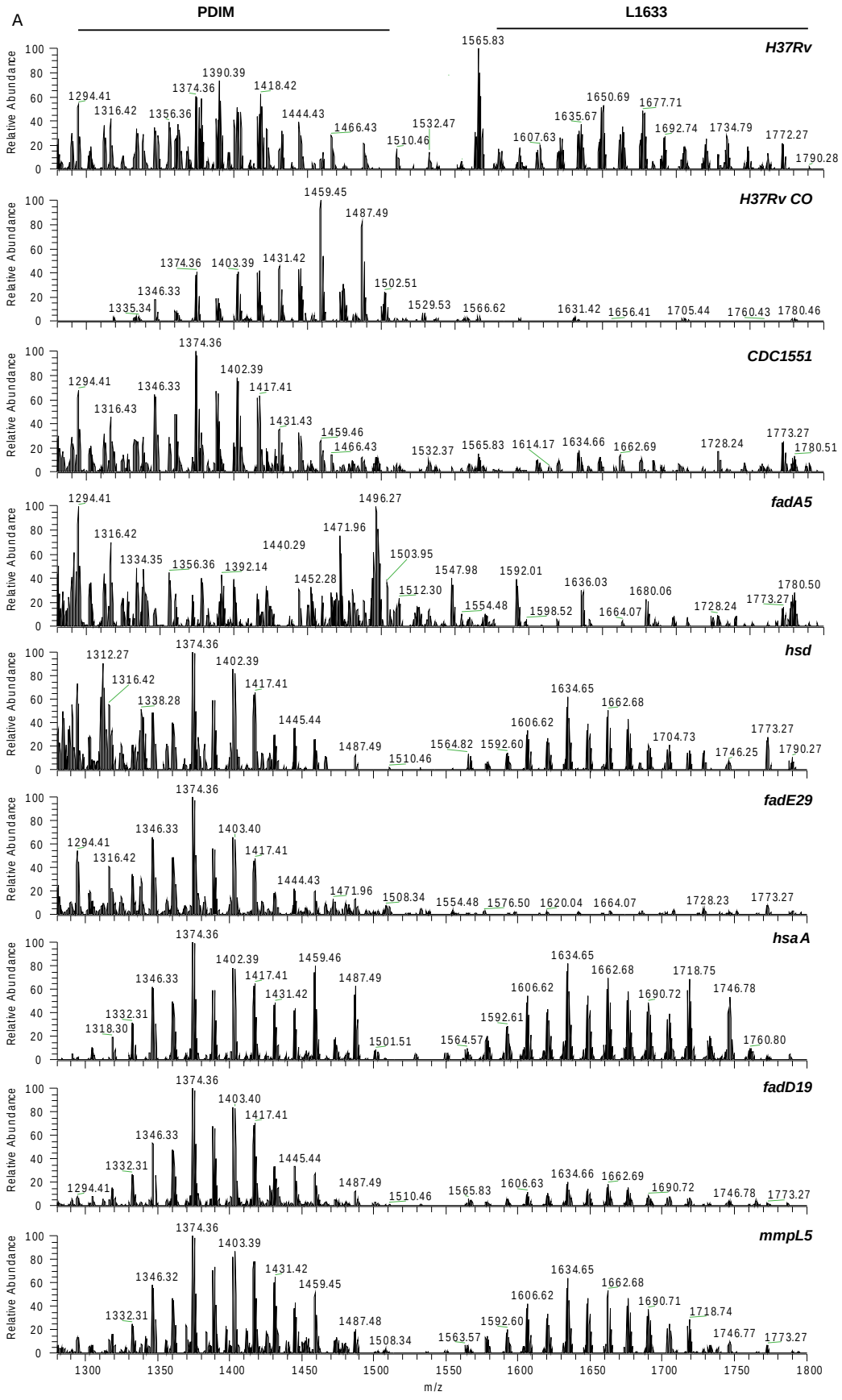
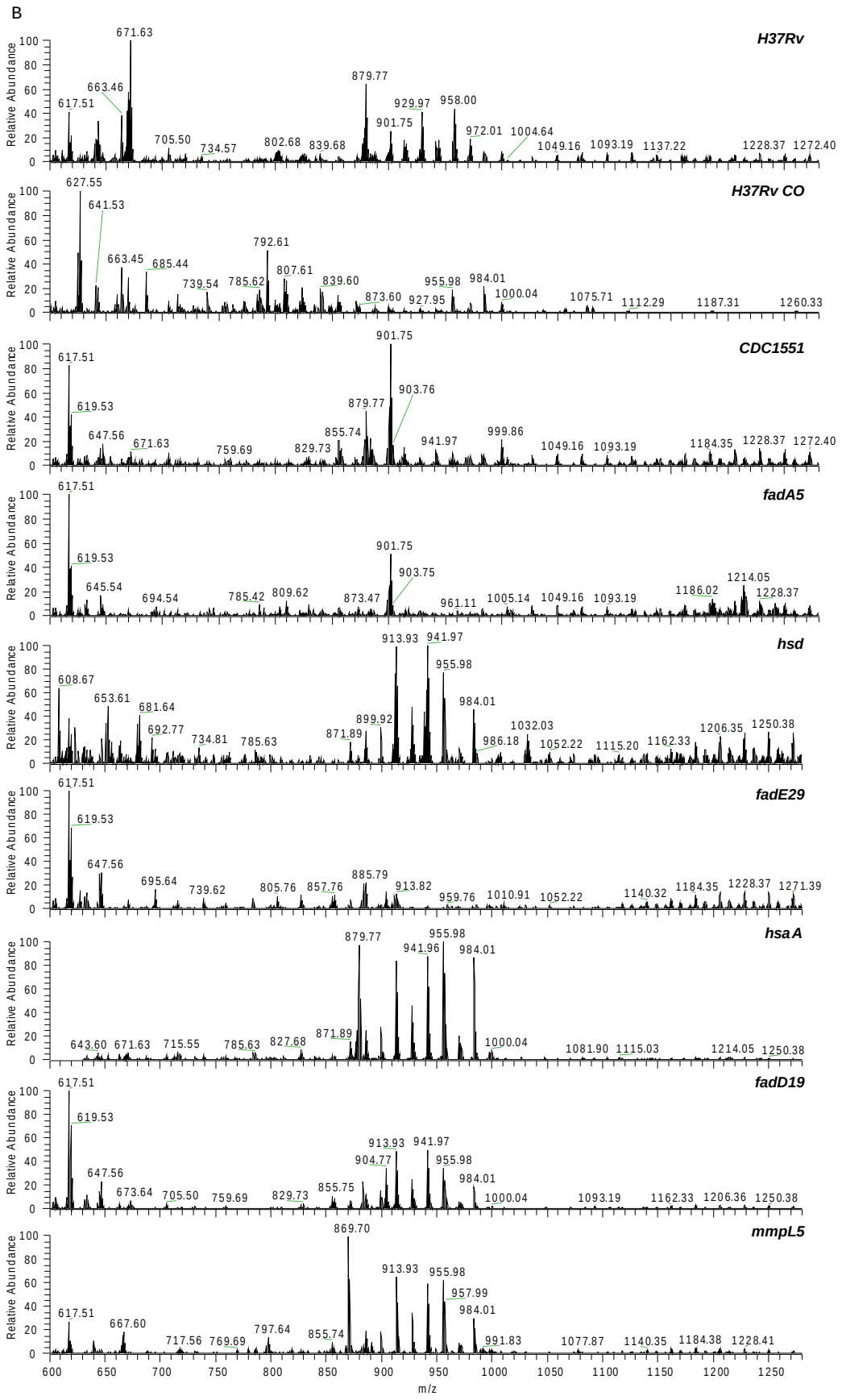

A
PDIM
L1633
H37Rv
H37Rv CO
CDC1551
fadA5
hsd
fadE29
hsaA
fadD19
mmpL5
Relative Abundance
m/z


B
H37Rv
671.63
663.46
617.51
879.77
929.97
958.00
901.75
705.50
734.57
802.68
839.68
972.01
1004.64
1049.16
1093.19
1137.22
1228.37
1272.40
H37Rv CO
627.55
641.53
663.45
685.44
792.61
807.61
739.54
785.62
839.60
873.60
927.95
955.98
984.01
1000.04
1075.71
1112.29
1187.31
1260.33
CDC1551
617.51
619.53
901.75
903.76
879.77
647.56
671.63
855.74
759.69
829.73
941.97
999.86
1049.16
1093.19
1184.35
1228.37
1272.40
fadA5
617.51
619.53
901.75
903.75
645.54
694.54
785.42
809.62
873.47
961.11
1005.14
1049.16
1093.19
1186.02
1214.05
1228.37
hsd
913.93
941.97
955.98
608.67
653.61
681.64
984.01
692.77
899.92
734.81
785.63
871.89
1032.03
986.18
1052.22
1115.20
1162.33
1206.35
1250.38
fadE29
617.51
619.53
647.56
695.64
739.62
805.76
857.76
885.79
913.82
959.76
1010.91
1052.22
1140.32
1184.35
1228.37
1271.39
hsaA
879.77
955.98
941.96
984.01
643.60
671.63
715.55
785.63
827.68
871.89
1000.04
1081.90
1115.03
1214.05
1250.38
fadD19
617.51
619.53
913.93
941.97
904.77
955.98
647.56
984.01
673.64
705.50
759.69
829.73
855.75
1000.04
1093.19
1162.33
1206.36
1250.38
mmpL5
869.70
913.93
955.98
957.99
617.51
984.01
667.60
717.56
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855.74
991.83
1077.87
1140.35
1184.38
1228.41
Relative Abundance
m/z

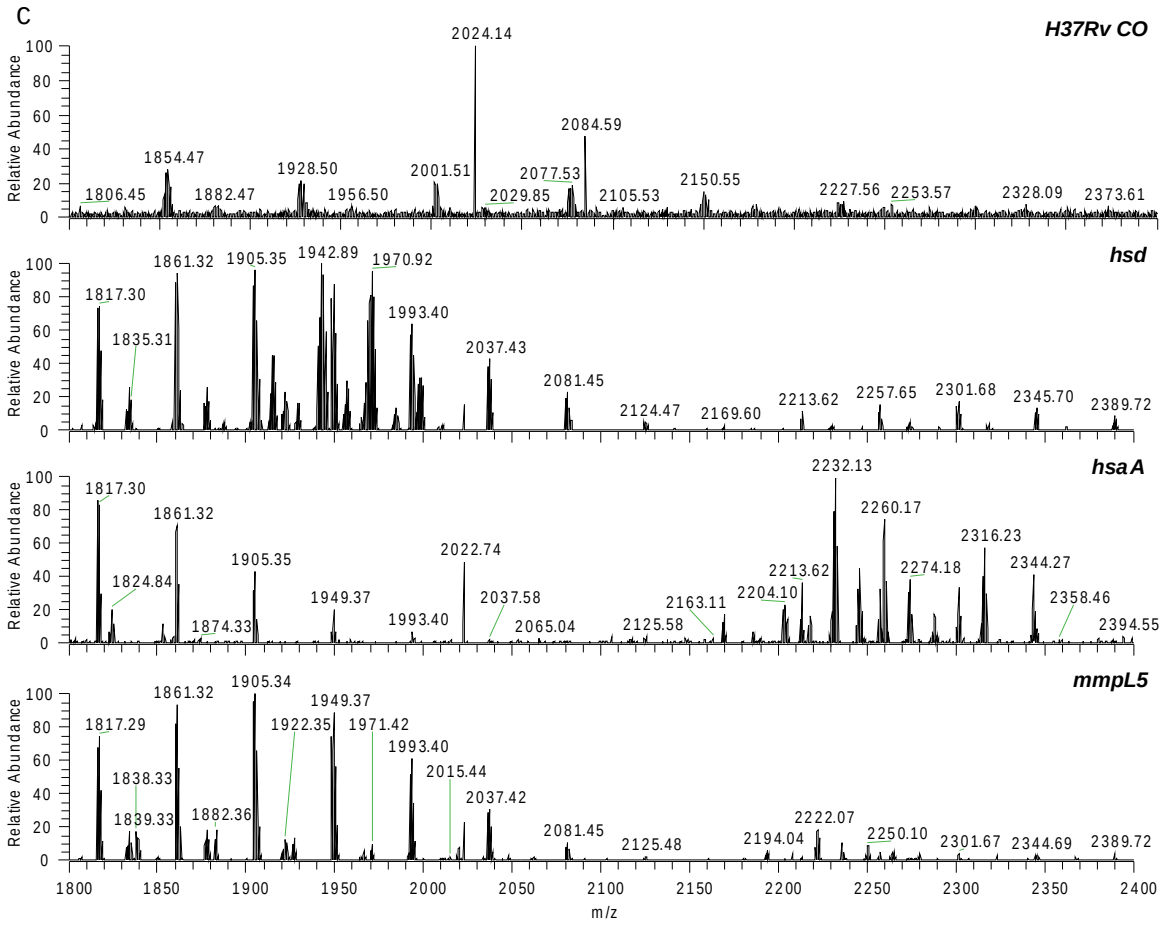

Figure 5-5. Mass spectrometric analysis of apolar lipids of wild-type H37Rv and CDC1551 *M. tb,* and mutants of *fadA5, hsd, fadE29, hsaA, fadD19* and *mmpL5,* grown in standard medium supplemented with 2.6 mM cholesterol solubilized in Tween-80 (1% w/v), and wild-type *H37Rv* grown using 2.6 mM cholesterol as the sole carbon source solubilized with tyloxapol (5% w/v) (H37Rv CO). A: mass range of 1280-1800 Th; B: mass range of 600-1280 Th; and C: mass range of 1800-2400 Th (only selected samples were shown due to the experimental setup.)

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Appendix

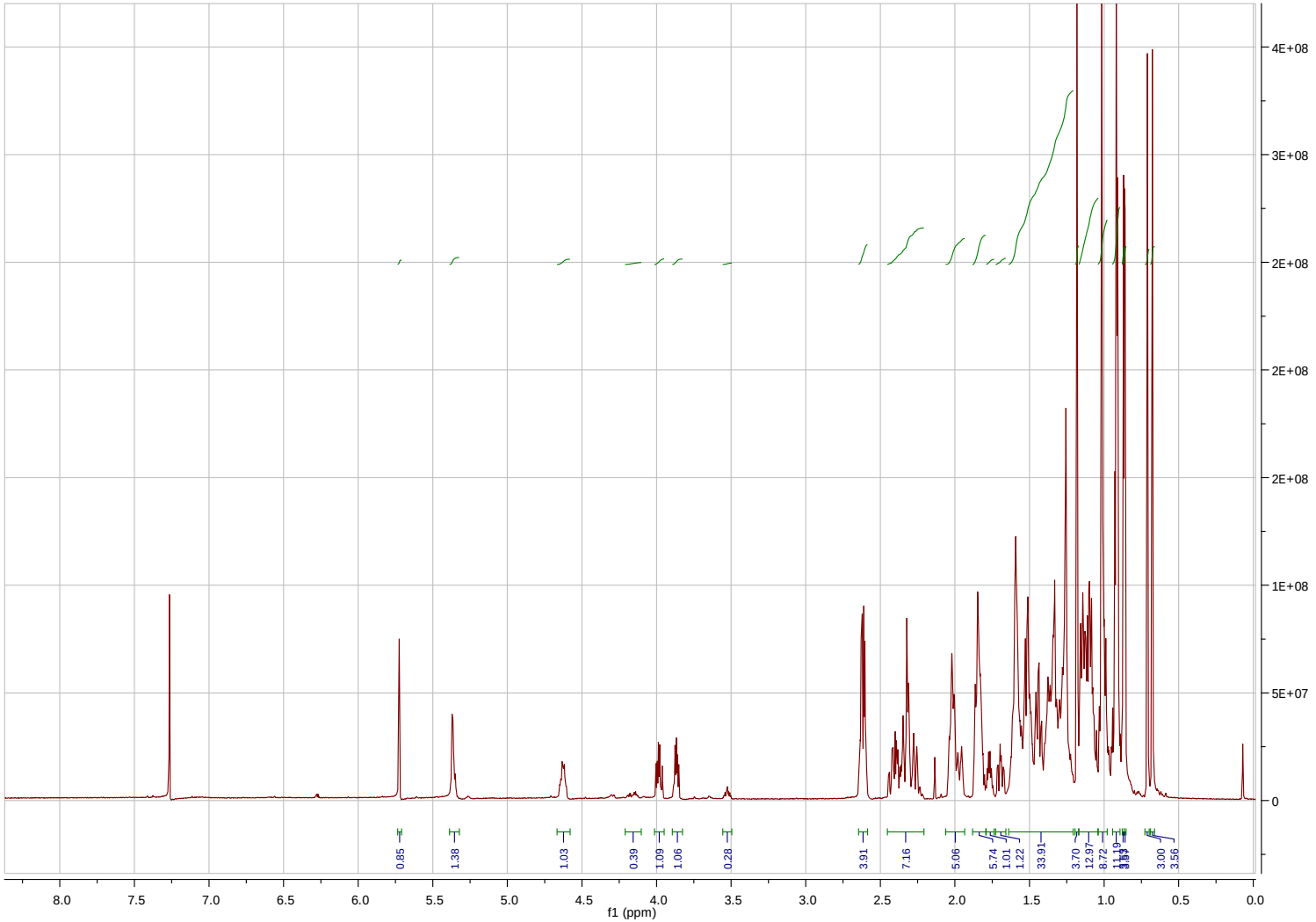

Figure 1S. ^{1}H NMR spectrum of **the** cholesterol-derived apolar lipid.

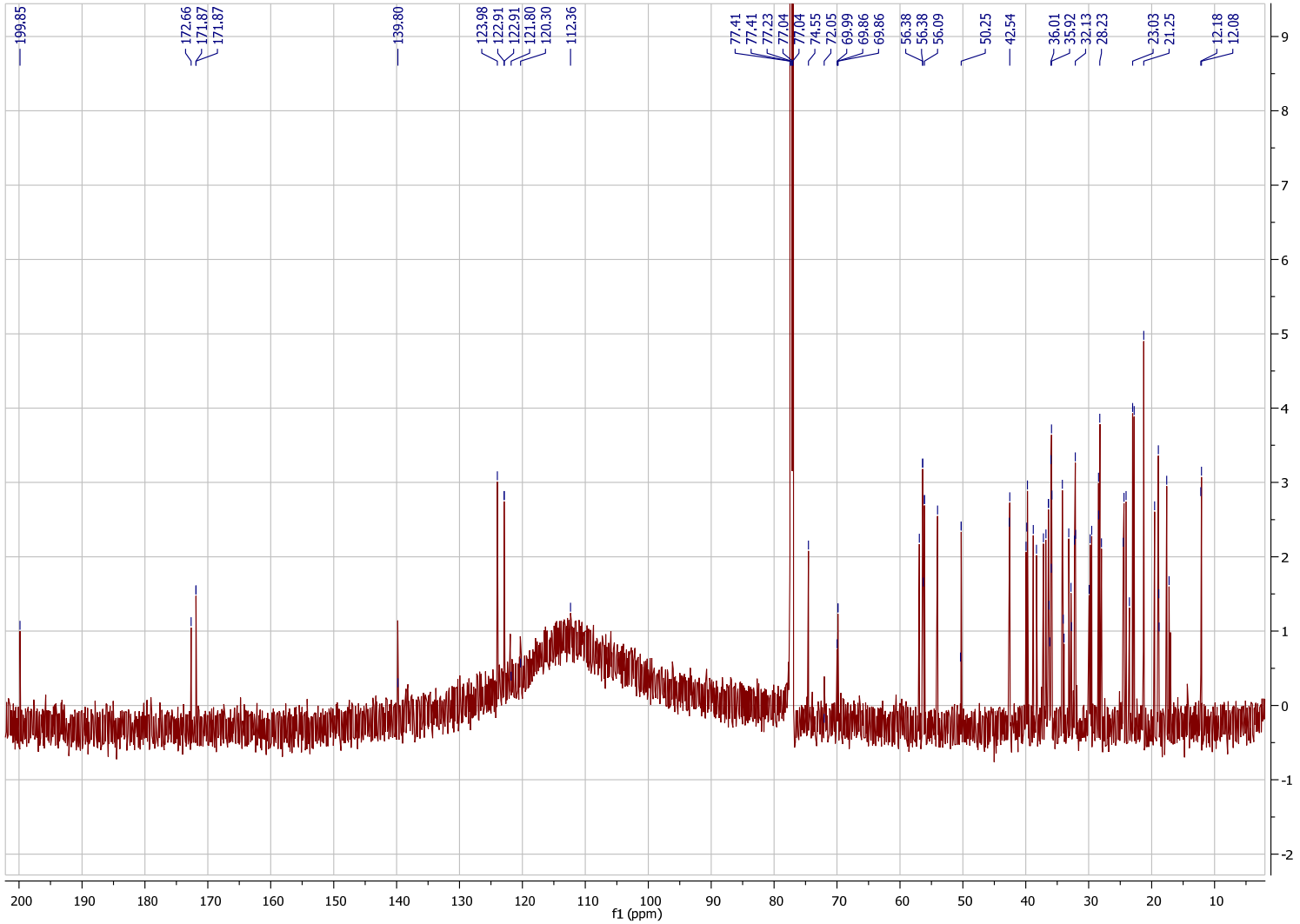

Figure 2S. ^{13}C NMR spectrum of the cholesterol-derived apolar lipid.

Table 1S. Summary of LC/MS experiments with *M. tb* samples. (All radioactive samples are marked in red.)

				Growth conditions															
#	Project name	File name	Mutants	Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
1	Xinxin	10Sep07 640-1A	control	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	N	Y	
2	Xinxin	10Sep07 640-2A	Rv	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	Y	Y	
3	Xinxin	10Sep07 640-3A	fadA5	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	Y	Y	
4	Xinxin	10Sep07 640-4A	echA19	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	Y	Y	
5	Xinxin	10Sep07 640-5A	hsd	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	N	Y	
6	Xinxin	10Sep07 640-6A	hsd comp1	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	Y	Y	
7	Xinxin	10Sep07 640-7A	hsd comp2	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	Y	Y	
8	Xinxin	10Sep07 640-8A	CDC	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	Y	Y	
9	Xinxin	10Sep07 640-1A-2	control	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	N	Y	
10	Xinxin	10Sep07 640-2A-2	Rv	Y	N	Y	N	N	24h	Y	N	Y	cholesterol	EtOAc	N	N	Y	Y	
11	Xinxin	24Sep2007 640-0B SIR	control	Y	N	Y	N	N	24h	N	N	N	dehydroepiandrosterone	C18:MeOH	N	N	N	N	
12	Xinxin	24Sep2007 640-1B SIR	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
13	Xinxin	24Sep2007 640-2B SIR	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
14	Xinxin	24Sep2007 640-3B SIR	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
15	Xinxin	24Sep2007 640-4B SIR	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
16	Xinxin	24Sep2007 640-5B SIR	hsd comp1	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
17	Xinxin	24Sep2007 640-6B SIR	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
18	Xinxin	24Sep2007 640-7B SIR	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
19	Xinxin	24Sep2007 640-4B-2 SIR	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
20	Xinxin	24Sep2007 640-6B-2 SIR	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
21	Xinxin	24Sep2007 640-0B TIC	control	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
22	Xinxin	24Sep2007 640-1B TIC	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
23	Xinxin	24Sep2007 640-2B TIC	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
24	Xinxin	24Sep2007 640-3B TIC	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
25	Xinxin	24Sep2007 640-4B TIC	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
26	Xinxin	24Sep2007 640-5B TIC	hsd comp1	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
27	Xinxin	24Sep2007 640-6B TIC	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
28	Xinxin	24Sep2007 640-7B TIC	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
29	Xinxin	24Sep2007 640-4B-2 TIC	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
30	Xinxin	24Sep2007 640-6B-2 TIC	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
31	Xinxin	28Sep2007 640-0B TIC	control	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
32	Xinxin	28Sep2007 640-1B TIC	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
33	Xinxin	28Sep2007 640-2B TIC	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
34	Xinxin	28Sep2007 640-3B TIC	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
35	Xinxin	28Sep2007 640-4B TIC	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
36	Xinxin	28Sep2007 640-5B TIC	hsd comp1	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
37	Xinxin	28Sep2007 640-6B TIC	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
38	Xinxin	24Sep2007 640-7B TIC	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
39	Xinxin	24Sep2007 640-4B-2 TIC	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
40	Xinxin	24Sep2007 640-6B-2 TIC	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18:MeOH	N	Y	N	N	
41	Xinxin	08Oct2007_693A0	control	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
42	Xinxin	08Oct2007_693A1	CDC	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
43	Xinxin	08Oct2007_693A2	fadE29	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
44	Xinxin	08Oct2007_693A4	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
45	Xinxin	08Oct2007_693A5	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
46	Xinxin	08Oct2007_693A6	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
47	Xinxin	08Oct2007_693A7	fadA5	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
48	Xinxin	08Oct2007_693A8	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
49	Xinxin	08Oct2007_693A9	CDC	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
50	Xinxin	08Oct2007_693A10	CDC	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
51	Xinxin	08Oct2007_693A11	fadD19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
52	Xinxin	08Oct2007_693A12	fadD19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
53	Xinxin	08Oct2007_693A13	fadE29	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
54	Xinxin	08Oct2007_693A14	fadE29	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	

#	Project name	File name	Mutants	Growth conditions					Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
				Cholesterol	Label	Tween-80	Tyloxypol													
55	Xinxin	08Oct2007_693A15	hsaA	N	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
56	Xinxin	08Oct2007_693A16	hsaA	Y	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
57	Xinxin	08Oct2007_693B0	control	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
58	Xinxin	08Oct2007_693B1	CDC	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
59	Xinxin	08Oct2007_693B2	fadE29	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
60	Xinxin	08Oct2007_693B3	Rv	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
61	Xinxin	08Oct2007_693B4	Rv	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
62	Xinxin	08Oct2007_693B5	echA19	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
63	Xinxin	08Oct2007_693B6	echA19	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
64	Xinxin	08Oct2007_693B7	fadA5	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
65	Xinxin	08Oct2007_693B8	fadA5	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
66	Xinxin	08Oct2007_693B9	CDC	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
67	Xinxin	08Oct2007_693B10	CDC	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
68	Xinxin	08Oct2007_693B11	fadD19	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
69	Xinxin	08Oct2007_693B12	fadD19	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
70	Xinxin	08Oct2007_693B13	fadE29	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
71	Xinxin	08Oct2007_693B14	fadE29	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
72	Xinxin	08Oct2007_693B15	hsaA	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
73	Xinxin	08Oct2007_693B16	hsaA	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
74	Xinxin	08Oct2007_693C0	control	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
75	Xinxin	08Oct2007_693C1	Rv	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
76	Xinxin	08Oct2007_693C2	Rv	Y	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
77	Xinxin	08Oct2007_693D0	control	N	N	Y	N	N		24h	Y	N	N	T:H	C18 with MeOH:H2O	N	N	N	Y	
78	Xinxin	08Oct2007_693D1	Rv	N	N	Y	N	N		24h	Y	N	N	T:H	C18 with MeOH:H2O	N	N	N	Y	
79	Xinxin	08Oct2007_693D2	Rv	Y	N	Y	N	N		24h	Y	N	N	T:H	C18 with MeOH:H2O	N	N	N	Y	
80	Xinxin	08Oct2007_693A3_1	Rv	N	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
81	Xinxin	09Oct2007_693C0	control	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
82	Xinxin	09Oct2007_693C1	Rv	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
83	Xinxin	09Oct2007_693C2	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
84	Xinxin	09Oct2007_6405A	hsd comp1	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	Y	Y	
85	Xinxin	09Oct2007_6406A	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	Y	Y	
86	Xinxin	09Oct2007_6405B	hsd comp1	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	Y	Y	
87	Xinxin	09Oct2007_6406B	hsd comp2	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	Y	Y	
88	Xinxin	12Oct2007_693A0	control	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
89	Xinxin	12Oct2007_693A1	CDC	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
90	Xinxin	12Oct2007_693A2	fadE29	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
91	Xinxin	12Oct2007_693A4	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
92	Xinxin	12Oct2007_693A5	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
93	Xinxin	12Oct2007_693A6	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
94	Xinxin	12Oct2007_693A7	fadA5	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
95	Xinxin	12Oct2007_693A8	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
96	Xinxin	12Oct2007_693A9	CDC	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
97	Xinxin	12Oct2007_693A10	CDC	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
98	Xinxin	12Oct2007_693A11	fadD19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
99	Xinxin	12Oct2007_693A12	fadD19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
100	Xinxin	12Oct2007_693A13	fadE29	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
101	Xinxin	12Oct2007_693A14	fadE29	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
102	Xinxin	12Oct2007_693A15	hsaA	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
103	Xinxin	12Oct2007_693A16	hsaA	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
104	Xinxin	12Oct2007_693B0	control	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	N	
105	Xinxin	12Oct2007_693B1	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
106	Xinxin	12Oct2007_693B2	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
107	Xinxin	12Oct2007_693B3	Rv	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
108	Xinxin	12Oct2007_693B4	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
109	Xinxin	12Oct2007_693B5	echA19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
110	Xinxin	12Oct2007_693B6	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
111	Xinxin	12Oct2007_693B7	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
112	Xinxin	12Oct2007_693B8	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
113	Xinxin	12Oct2007_693B9	CDC	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
114	Xinxin	12Oct2007_693B10	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
115	Xinxin	12Oct2007_693B11	fadD19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
116	Xinxin	12Oct2007_693B12	fadD19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
117	Xinxin	12Oct2007_693B13	fadE29	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
118	Xinxin	12Oct2007_693B14	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
119	Xinxin	12Oct2007_693B15	hsaA	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
120	Xinxin	12Oct2007_693B16	hsaA	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
121	Xinxin	12Oct2007_693C0	control	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
122	Xinxin	12Oct2007_693C1	Rv	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
123	Xinxin	12Oct2007_693C2	RV	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
124	Xinxin	12Oct2007_693D0	control	N	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:H2O	N	N	N	Y	
125	Xinxin	12Oct2007_693D1	Rv	N	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:H2O	N	N	N	Y	
126	Xinxin	12Oct2007_693D2	RV	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:H2O	N	N	N	Y	
127	Xinxin	12Oct2007_693A3_1	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
128	Xinxin	16Oct2007_693B0	control	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
129	Xinxin	16Oct2007_693B1	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
130	Xinxin	16Oct2007_693B2	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
131	Xinxin	16Oct2007_693B3	Rv	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
132	Xinxin	16Oct2007_693A0	control	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
133	Xinxin	16Oct2007_693A1	CDC	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
134	Xinxin	16Oct2007_693A2	fadE29	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
135	Xinxin	16Oct2007_693A4	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:H2O	N	N	N	Y	
136	Xinxin	17Oct2007_693B4	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	Y	N	N	
137	Xinxin	17Oct2007_693B7	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
138	Xinxin	17Oct2007_693B11	fadD19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
139	Xinxin	17Oct2007_693C1	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
140	Xinxin	18Oct2007_693C1	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
141	Xinxin	23Oct2007_693B11	fadD19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
142	Xinxin	23Oct2007_693B11_2	fadD19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
143	Xinxin	23Oct2007_693C0	control	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
144	Xinxin	23Oct2007_693C0_2	control	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
145	Xinxin	23Oct2007_693C1	Rv	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
146	Xinxin	23Oct2007_693C1_2	Rv	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
147	Xinxin	23Oct2007_693C2	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
148	Xinxin	23Oct2007_693C2_2	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:H2O	N	N	N	Y	
149	Xinxin	26Oct2007_693B11	fadD19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
150	Xinxin	26Oct2007_693B11_2	fadD19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
151	Xinxin	26Oct2007_693B7	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
152	Xinxin	26Oct2007_693C1	Rv	N	N	Y	N	N	24h	Y	N	N	R:N	C18 with MeOH:H2O	N	N	N	Y	
153	Xinxin	01Nov2007_693B10	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
154	Xinxin	01Nov2007_693B10_1	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:H2O	N	N	N	N	
155	Xinxin	19Nov2007_736_1_1	Rv	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
156	Xinxin	19Nov2007_736_1_2	Rv	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
157	Xinxin	19Nov2007_736_1_3	Rv	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
158	Xinxin	19Nov2007_736_1_4	Rv	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
159	Xinxin	19Nov2007_736_1_5	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
160	Xinxin	19Nov2007_736_1_6	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
161	Xinxin	19Nov2007_736_1_7	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
162	Xinxin	19Nov2007_736_1_8	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
163	Xinxin	19Nov2007_736_1_9	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
164	Xinxin	19Nov2007_736_1_10	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
165	Xinxin	19Nov2007_736_2_1	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
166	Xinxin	19Nov2007_736_2_2	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	

#	Project name	File name	Mutants	Growth conditions					Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
				Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates											
167	Xinxin	19Nov2007_736_2_3	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
168	Xinxin	19Nov2007_736_2_4	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
169	Xinxin	19Nov2007_736_2_5	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
170	Xinxin	19Nov2007_736_2_6	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
171	Xinxin	19Nov2007_736_2_7	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
172	Xinxin	19Nov2007_736_2_8	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
173	Xinxin	19Nov2007_736_2_9	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
174	Xinxin	19Nov2007_736_2_10	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
175	Xinxin	19Nov2007_736_3_1	echA19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
176	Xinxin	19Nov2007_736_3_2	echA19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
177	Xinxin	19Nov2007_736_3_3	echA19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
178	Xinxin	19Nov2007_736_3_4	echA19	N	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
179	Xinxin	19Nov2007_736_3_5	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
180	Xinxin	19Nov2007_736_3_6	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
181	Xinxin	19Nov2007_736_3_7	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
182	Xinxin	19Nov2007_736_3_8	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
183	Xinxin	19Nov2007_736_3_9	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
184	Xinxin	19Nov2007_736_3_10	echA19	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
185	Xinxin	19Nov2007_736_4_1	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
186	Xinxin	19Nov2007_736_4_2	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
187	Xinxin	19Nov2007_736_4_3	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
188	Xinxin	19Nov2007_736_4_4	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
189	Xinxin	19Nov2007_736_4_5	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
190	Xinxin	19Nov2007_736_4_6	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
191	Xinxin	19Nov2007_736_4_7	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
192	Xinxin	19Nov2007_736_4_8	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
193	Xinxin	19Nov2007_736_4_9	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
194	Xinxin	19Nov2007_736_4_10	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	

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				Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates											
195	Xinxin	19Nov2007_736_5_1	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
196	Xinxin	19Nov2007_736_5_2	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
197	Xinxin	19Nov2007_736_5_3	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
198	Xinxin	19Nov2007_736_5_4	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
199	Xinxin	19Nov2007_736_5_5	fadA5	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
200	Xinxin	19Nov2007_736_5_6	fadA5	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
201	Xinxin	19Nov2007_736_5_8	fadA5	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
202	Xinxin	19Nov2007_736_5_9	fadA5	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
203	Xinxin	19Nov2007_736_5_10	fadA5	N	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
204	Xinxin	19Nov2007_736_6_1	fadA5	N	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
205	Xinxin	19Nov2007_736_6_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
206	Xinxin	19Nov2007_736_6_3	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
207	Xinxin	19Nov2007_736_6_4	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	N	N	N	
208	Xinxin	19Nov2007_736_6_5	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
209	Xinxin	19Nov2007_736_6_6	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
210	Xinxin	19Nov2007_736_6_7	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
211	Xinxin	19Nov2007_736_6_8	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
212	Xinxin	19Nov2007_736_6_9	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
213	Xinxin	19Nov2007_736_6_10	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	Y	
214	Xinxin	20Nov2007_737_1	Rv	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
215	Xinxin	20Nov2007_737_2	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
216	Xinxin	20Nov2007_737_3	echA19	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
217	Xinxin	20Nov2007_737_4	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
218	Xinxin	20Nov2007_737_5	fadA5	N	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
219	Xinxin	20Nov2007_737_6	fadA5	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
220	Xinxin	21Nov2007_695_E1	Rv	N	N	Y	N	N	24h	Y	N	N	cholesterol 14C	C18 with MeOH:IPA	N	N	N	Y	
221	Xinxin	21Nov2007_695_E2	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol 14C	C18 with MeOH:IPA	N	N	N	Y	
222	Xinxin	26Nov2007_737_7	Rv	N	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	Y	N	N	

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				Cholesterol	Label	Tween-80	Tyloxypol													
223	Xinxin	26Nov2007_737_8	Rv	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
224	Xinxin	26Nov2007_737_9	echA19	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
225	Xinxin	26Nov2007_737_11	fadA5	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	m/z 255,273
226	Xinxin	26Nov2007_737_12	fadA5	Y	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	m/z 255,273
227	Xinxin	26Nov2007_737_13	control	N	N	Y	N	N		24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
228	Xinxin	26Nov2007_736_1_1	Rv	N	N	Y	N	N		25h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
229	Xinxin	26Nov2007_736_1_3	echA19	N	N	Y	N	N		26h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
230	Xinxin	26Nov2007_736_1_1and3re	Rv echA19	N	N	Y	N	N		27h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	N	N	
231	Xinxin	27Nov2007_737_1	Rv	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
232	Xinxin	27Nov2007_737_2	Rv	Y	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
233	Xinxin	27Nov2007_737_3	echA19	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
234	Xinxin	27Nov2007_737_4	echA19	Y	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
235	Xinxin	27Nov2007_737_5	fadA5	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
236	Xinxin	27Nov2007_737_6	fadA5	Y	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
237	Xinxin	28Nov2007_737_1	Rv	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
238	Xinxin	03Dec2007_737_1_1	Rv	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
239	Xinxin	03Dec2007_737_1_2	Rv	Y	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
240	Xinxin	03Dec2007_737_1_3	echA19	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
241	Xinxin	03Dec2007_737_1_4	echA19	Y	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
242	Xinxin	03Dec2007_737_1_5	fadA5	N	N	Y	N	N		24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
243	XinxinDec2007	16Dec07_763_1_1	Rv	Y	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
244	XinxinDec2007	16Dec07_763_1_2	Rv	Y	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
245	XinxinDec2007	16Dec07_763_1_3	Rv	Y	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
246	XinxinDec2007	16Dec07_763_1_4	Rv	Y	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
247	XinxinDec2007	16Dec07_763_1_6	Rv	Y	N	Y	N	N		24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	detector contaminated
248	XinxinDec2007	16Dec07_763_1_7	Rv	Y	N	Y	N	N		24h	Y	N	N	T	C18 with MeOH:IPA	N	N	N	N	detector contaminated
249	XinxinDec2007	16Dec07_763_2_2	echA19	Y	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
250	XinxinDec2007	16Dec07_763_2_4	echA19	Y	N	Y	N	N		24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated

#	Project name	File name	Mutants	Growth conditions		Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
				Cholesterol	Label														
251	XinxinDec2007	16Dec07_763_2_6	echA19	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	detector contaminated
252	XinxinDec2007	16Dec07_763_3_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
253	XinxinDec2007	16Dec07_763_3_4	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
254	XinxinDec2007	16Dec07_763_3_6	fadA5	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	detector contaminated
255	XinxinDec2007	16Dec07_763_3_7	fadA5	Y	N	Y	N	N	24h	Y	N	N	T	C18 with MeOH:IPA	N	N	N	N	detector contaminated
256	XinxinDec2007	29Dec07_763_1_1	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
257	XinxinDec2007	29Dec07_763_1_2	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
258	XinxinDec2007	29Dec07_763_1_3	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
259	XinxinDec2007	29Dec07_763_1_4	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
260	XinxinDec2007	29Dec07_763_1_6	Rv	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	detector contaminated
261	XinxinDec2007	29Dec07_763_1_7	Rv	Y	N	Y	N	N	24h	Y	N	N	T	C18 with MeOH:IPA	N	N	N	N	detector contaminated
262	XinxinDec2007	29Dec07_763_2_2	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
263	XinxinDec2007	29Dec07_763_2_4	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
264	XinxinDec2007	29Dec07_763_2_6	echA19	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	detector contaminated
265	XinxinDec2007	29Dec07_763_3_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
266	XinxinDec2007	29Dec07_763_3_4	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
267	XinxinDec2007	29Dec07_763_3_6	fadA5	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	detector contaminated
268	XinxinDec2007	29Dec07_763_3_7	fadA5	Y	N	Y	N	N	24h	Y	N	N	T	C18 with MeOH:IPA	N	N	N	N	detector contaminated
269	XinxinDec2007	29Dec07_763_1_9	Rv	Y	N	Y	N	N	24h	Y	N	N	C14-cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
270	XinxinDec2007	29Dec07_763_2_9	echA19	Y	N	Y	N	N	24h	Y	N	N	C14-cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
271	XinxinDec2007	29Dec07_763_3_9	fadA5	Y	N	Y	N	N	24h	Y	N	N	C14-cholesterol	C18 with MeOH:IPA	N	N	N	N	detector contaminated
272	XinxinDec2007	29Dec07_763_1_8	Rv	Y	N	Y	N	N	24h	Y	N	N	H3-dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	detector contaminated
273	XinxinDec2007	06Feb08_777_1_1	Rv	Y	N	Y	N	N	24h	Y	N	N	4-C14 cholesterol	C18 with MeOH	N	N	Y	Y	
274	XinxinDec2007	06Feb08_777_1_1_2	Rv	Y	N	Y	N	N	24h	Y	N	N	4-C14 cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
275	XinxinDec2007	06Feb08_777_2_1	echA19	Y	N	Y	N	N	24h	Y	N	N	4-C14 cholesterol	C18 with MeOH	N	N	Y	Y	
276	XinxinDec2007	06Feb08_777_2_1_2	echA19	Y	N	Y	N	N	24h	Y	N	N	4-C14 cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
277	XinxinDec2007	06Feb08_777_3_1	fadA5	Y	N	Y	N	N	24h	Y	N	N	4-C14 cholesterol	C18 with MeOH	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
278	XinxinDec2007	06Feb08_777_3_1_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	4-C14 cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
279	XinxinDec2007		Rv	Y	N	Y	N	N	24h	Y	N	N	26-C14 cholesterol	C18 with MeOH	N	N	Y	Y	
280	XinxinDec2007	06Feb08_777_1_2_2	Rv	Y	N	Y	N	N	24h	Y	N	N	26-C14 cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
281	XinxinDec2007	06Feb08_777_2_2	echA19	Y	N	Y	N	N	24h	Y	N	N	26-C14 cholesterol	C18 with MeOH	N	N	Y	Y	
282	XinxinDec2007	06Feb08_777_2_2_2	echA19	Y	N	Y	N	N	24h	Y	N	N	26-C14 cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
283	XinxinDec2007	06Feb08_777_3_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	26-C14 cholesterol	C18 with MeOH	N	N	Y	Y	
284	XinxinDec2007	06Feb08_777_3_2_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	26-C14 cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
285	XinxinDec2007	06Feb08_777_2_4	echA19	Y	N	Y	N	N	24h	Y	N	N	H3-dehydroepiandrosterone	C18 with MeOH	N	N	N	N	
286	XinxinDec2007	06Feb08_777_2_4_2	echA19	Y	N	Y	N	N	24h	Y	N	N	H3-dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
287	XinxinDec2007	06Feb08_777_3_4	fadA5	Y	N	Y	N	N	24h	Y	N	N	H3-dehydroepiandrosterone	C18 with MeOH	N	N	N	N	
288	XinxinDec2007	06Feb08_777_3_4_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	H3-dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
289	XinxinDec2007	07Feb08_777_1_3	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
290	XinxinDec2007	07Feb08_777_2_3	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
291	XinxinDec2007	07Feb08_777_3_3	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
292	XinxinDec2007	07Feb08_777_1_5	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
293	XinxinDec2007	07Feb08_777_2_5	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
294	XinxinDec2007	07Feb08_777_3_5	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	N	
295	XinxinDec2007	13Feb08_777_1_3	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
296	XinxinDec2007	13Feb08_777_2_3	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
297	XinxinDec2007	13Feb08_777_3_3	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
298	XinxinDec2007	13Feb08_777_1_5	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	Y	Y	
299	XinxinDec2007	13Feb08_777_2_5	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	Y	Y	
300	XinxinDec2007	13Feb08_777_3_5	fadA5	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	Y	Y	
301	XinxinDec2007	16Feb08_763_1_3	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
302	XinxinDec2007	16Feb08_763_1_6	Rv	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	Y	Y	
303	XinxinDec2007	16Feb08_777_7c	Rv	Y	C14	Y	N	N	14h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
304	XinxinDec2007	16Feb08_777_7c2	Rv	Y	C14	Y	N	N	14h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	

#	Project name	File name	Mutants	Growth conditions					Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
				Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates											
305	XinxinDec2007	16Feb08_777_8c	echA19	Y	C14	Y	N	N	14h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
306	XinxinDec2007	16Feb08_777_8c2	echA19	Y	C14	Y	N	N	14h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
307	XinxinDec2007	16Feb08_777_9c	fadA5	Y	C14	Y	N	N	14h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
308	XinxinDec2007	16Feb08_777_9c2	fadA5	Y	C14	Y	N	N	14h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
309	XinxinDec2007	22Feb08_763_1_3	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	
310	XinxinDec2007	22Feb08_763_1_6	Rv	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	
311	XinxinDec2007	22Feb08_763_1_7	Rv	Y	N	Y	N	N	24h	Y	N	N	T	C18 with MeOH:IPA	N	N	N	N	
312	XinxinDec2007	22Feb08_763_2_4	echA19	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	
313	XinxinDec2007	22Feb08_763_2_6	echA19	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	
314	XinxinDec2007	22Feb08_763_3_4	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	N	N	
315	XinxinDec2007	22Feb08_763_3_6	fadA5	Y	N	Y	N	N	24h	Y	N	N	R	C18 with MeOH:IPA	N	N	N	N	
316	XinxinDec2007	22Feb08_763_3_7	fadA5	Y	N	Y	N	N	24h	Y	N	N	T	C18 with MeOH:IPA	N	N	N	N	
317	XinxinDec2007	11March08_800_1_1	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH	N	N	N	N	
318	XinxinDec2007	11March08_800_2_1	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH	N	N	N	N	
319	XinxinDec2007	11March08_800_3_1	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH	N	N	N	N	
320	XinxinDec2007	11March08_800_4_1	Rv	Y	N	Y	N	N	24h	Y	N	N	control	C18 with MeOH	N	N	N	N	
321	XinxinDec2007	11March08_800_5_1	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH	N	N	N	N	
322	XinxinDec2007	11March08_800_6_1	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH	N	N	N	N	
323	XinxinDec2007	11March08_800_7_1	Rv	Y	N	Y	N	N	24h	Y	N	N	cortisol	C18 with MeOH	N	N	N	N	
324	XinxinDec2007	11March08_800_1_2	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
325	XinxinDec2007	11March08_800_2_2	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
326	XinxinDec2007	11March08_800_3_2	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
327	XinxinDec2007	11March08_800_4_2	Rv	Y	N	Y	N	N	24h	Y	N	N	control	C18 with MeOH:IPA	N	N	N	N	
328	XinxinDec2007	11March08_800_5_2	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepian drosterone	C18 with MeOH:IPA	N	Y	Y	Y	
329	XinxinDec2007	11March08_800_6_2	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	progesterone, diendione
330	XinxinDec2007	11March08_800_7_2	Rv	Y	N	Y	N	N	24h	Y	N	N	cortisol	C18 with MeOH:IPA	N	N	Y	Y	
331	XinxinDec2007	12March08_800_1_2	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
332	XinxinDec2007	16March08_800_3_2	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions					Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
				Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates											
333	XinxinDec2007	16March08_800_4_2	Rv	Y	N	Y	N	N	24h	Y	N	N	control	C18 with MeOH:IPA	N	N	N	N	
334	XinxinDec2007	16March08_800_5_2	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	Y	Y	Y	
335	XinxinDec2007	16March08_800_6_2	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	progesterone, diendione
336	XinxinDec2007	16March08_800_7_2	Rv	Y	N	Y	N	N	24h	Y	N	N	cortisol	C18 with MeOH:IPA	N	N	Y	Y	
337	XinxinDec2007	18March08_800_5c	Rv	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
338	XinxinDec2007	18March08_800_6c	Rv	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
339	XinxinDec2007	18March08_800_7c	Rv	Y	C14	Y	N	N	72h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
340	XinxinDec2007	19March08_800_5c	Rv	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
341	XinxinDec2007	19March08_800_6c	Rv	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
342	XinxinDec2007	19March08_800_7c	Rv	Y	C14	Y	N	N	72h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
343	XinxinDec2007	19March08_800_6c2	Rv	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
344	XinxinDec2007	19March08_800_7c2	Rv	Y	C14	Y	N	N	72h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
345	XinxinDec2007	20March08_800_5c	Rv	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
346	XinxinDec2007	20March08_800_6c	Rv	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
347	XinxinDec2007	20March08_800_7c	Rv	Y	C14	Y	N	N	72h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
348	XinxinDec2007	20March08_800_6c2	Rv	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
349	XinxinDec2007	20March08_800_7c2	Rv	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
350	XinxinDec2007	21March08_800_7c2	Rv	Y	C14	Y	N	N	72h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
351	XinxinApril2008	02April2008_815_1_1b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
352	XinxinApril2008	02April2008_815_1_4b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
353	XinxinApril2008	02April2008_815_2_1b	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
354	XinxinApril2008	02April2008_815_2_4b	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
355	XinxinApril2008	02April2008_815_3_1b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
356	XinxinApril2008	02April2008_815_3_4b	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
357	XinxinApril2008	02April2008_815_4_1b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
358	XinxinApril2008	02April2008_815_4_4b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
359	XinxinApril2008	02April2008_815_5_1b	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
360	XinxinApril2008	02April2008_815_5_4b	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
361	XinxinApril2008	02April2008_815_6_1b	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
362	XinxinApril2008	02April2008_815_6_4b	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
363	XinxinApril2008/	02April2008_815_1_2b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
364	XinxinApril2008	02April2008_815_1_5b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
365	XinxinApril2008	02April2008_815_2_2b	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
366	XinxinApril2008	02April2008_815_2_5b	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
367	XinxinApril2008	02April2008_815_3_2b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
368	XinxinApril2008	02April2008_815_3_5b	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
369	XinxinApril2008	02April2008_815_4_2b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
370	XinxinApril2008	02April2008_815_4_5b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
371	XinxinApril2008	02April2008_815_5_2b	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
372	XinxinApril2008	02April2008_815_5_5b	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
373	XinxinApril2008	02April2008_815_6_2b	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
374	XinxinApril2008	02April2008_815_6_5b	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
375	XinxinApril2008	02April2008_815_1_3b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
376	XinxinApril2008	02April2008_815_1_6b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
377	XinxinApril2008	02April2008_815_2_3b	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
378	XinxinApril2008	02April2008_815_2_6b	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
379	XinxinApril2008	02April2008_815_3_3b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
380	XinxinApril2008	02April2008_815_3_6b	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
381	XinxinApril2008	02April2008_815_4_3b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
382	XinxinApril2008	02April2008_815_4_6b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
383	XinxinApril2008	02April2008_815_5_3b	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
384	XinxinApril2008	02April2008_815_5_6b	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
385	XinxinApril2008	02April2008_815_6_3b	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
386	XinxinApril2008	02April2008_815_6_6b	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
387	XinxinApril2008	02April2008_815_1_7b	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
388	XinxinApril2008	02April2008_815_1_10b	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated

#	Project name	File name	Growth conditions						Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
			Mutants	Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates											
389	XinxinApril2008	02April2008_815_2_7b	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
390	XinxinApril2008	02April2008_815_2_10b	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
391	XinxinApril2008	02April2008_815_3_7b	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
392	XinxinApril2008	02April2008_815_3_10b	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
393	XinxinApril2008	02April2008_815_4_7b	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
394	XinxinApril2008	02April2008_815_4_10b	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
395	XinxinApril2008	02April2008_815_5_7b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
396	XinxinApril2008	02April2008_815_5_10b	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
397	XinxinApril2008	02April2008_815_6_7b	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
398	XinxinApril2008	02April2008_815_6_10b	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
399	XinxinApril2008	02April2008_815_1_8b	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
400	XinxinApril2008	02April2008_815_1_11b	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
401	XinxinApril2008	02April2008_815_2_8b	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
402	XinxinApril2008	02April2008_815_2_11b	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
403	XinxinApril2008	02April2008_815_3_8b	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
404	XinxinApril2008	02April2008_815_3_11b	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
405	XinxinApril2008	02April2008_815_4_8b	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
406	XinxinApril2008	02April2008_815_4_11b	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
407	XinxinApril2008	02April2008_815_5_8b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
408	XinxinApril2008	02April2008_815_5_11b	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
409	XinxinApril2008	02April2008_815_6_8b	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
410	XinxinApril2008	02April2008_815_6_11b	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
411	XinxinApril2008	02April2008_815_1_9b	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
412	XinxinApril2008	02April2008_815_1_12b	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
413	XinxinApril2008	02April2008_815_2_9b	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
414	XinxinApril2008	02April2008_815_2_12b	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
415	XinxinApril2008	02April2008_815_3_9b	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
416	XinxinApril2008	02April2008_815_3_12b	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
417	XinxinApril2008	02April2008_815_4_9b	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
418	XinxinApril2008	02April2008_815_4_12b	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	SQ detector contaminated
419	XinxinApril2008	02April2008_815_5_9b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
420	XinxinApril2008	02April2008_815_5_12b	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
421	XinxinApril2008	02April2008_815_6_9b	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
422	XinxinApril2008	02April2008_815_6_12b	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
423	XinxinApril2008	02April2008_815_3_14b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
424	XinxinApril2008	02April2008_815_3_16b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
425	XinxinApril2008	02April2008_815_3_18b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
426	XinxinApril2008	02April2008_815_3_20b	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
427	XinxinApril2008	02April2008_815_3_21b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
428	XinxinApril2008	02April2008_815_3_22b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
429	XinxinApril2008	02April2008_815_3_23b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
430	XinxinApril2008	02April2008_815_3_24b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
431	XinxinApril2008	02April2008_815_3_25b	echA19	Y	N	Y	N	N	24h	Y	N	N	control	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
432	XinxinApril2008	02April2008_815_4_13b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
433	XinxinApril2008	02April2008_815_4_15b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
434	XinxinApril2008	02April2008_815_4_17b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
435	XinxinApril2008	02April2008_815_4_19b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	SQ detector contaminated
436	XinxinApril2008/05April2008methanolext	02April2008_815_1_1	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
437	XinxinApril2008/05April2008methanolext	02April2008_815_1_4	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
438	XinxinApril2008/05April2008methanolext	02April2008_815_2_1	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
439	XinxinApril2008/05April2008methanolext	02April2008_815_2_4	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
440	XinxinApril2008/05April2008methanolext	02April2008_815_3_1	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
441	XinxinApril2008/05April2008methanolext	02April2008_815_3_4	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
442	XinxinApril2008/05April2008methanolext	02April2008_815_4_1	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
443	XinxinApril2008methanolext	02April2008_815_4_4	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	
444	XinxinApril2008methanolext	02April2008_815_5_1	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
445	XinxinApril2008methanolext	02April2008_815_5_4	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
446	XinxinApril2008methanolext	02April2008_815_6_1	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
447	XinxinApril2008methanolext	02April2008_815_6_4	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
448	XinxinApril2008methanolext	02April2008_815_1_2	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
449	XinxinApril2008methanolext	02April2008_815_1_5	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
450	XinxinApril2008methanolext	02April2008_815_2_2	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
451	XinxinApril2008methanolext	02April2008_815_2_5	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
452	XinxinApril2008methanolext	02April2008_815_3_2	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
453	XinxinApril2008methanolext	02April2008_815_3_5	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
454	XinxinApril2008methanolext	02April2008_815_4_2	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	
455	XinxinApril2008methanolext	02April2008_815_4_5	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	
456	XinxinApril2008methanolext	02April2008_815_5_2	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
457	XinxinApril2008methanolext	02April2008_815_5_5	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
458	XinxinApril2008methanolext	02April2008_815_6_2	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	
459	XinxinApril2008methanolext	02April2008_815_6_5	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	
460	XinxinApril2008methanolext	02April2008_815_1_3	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
461	XinxinApril2008methanolext	02April2008_815_1_6	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
462	XinxinApril2008methanolext	02April2008_815_2_3	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
463	XinxinApril2008methanolext	02April2008_815_2_6	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
464	XinxinApril2008methanolext	02April2008_815_3_3	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
465	8/05April2008methanolext	02April2008_815_3_6	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
466	XinxinApril2008methanolext	02April2008_815_4_3	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	
467	XinxinApril2008methanolext	02April2008_815_4_6	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	
468	XinxinApril2008methanolext	02April2008_815_5_3	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
469	XinxinApril2008methanolext	02April2008_815_5_6	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
470	XinxinApril2008methanolext	02April2008_815_6_3	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
471	XinxinApril2008methanolext	02April2008_815_6_6	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
472	XinxinApril2008methanolext	02April2008_815_1_7	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
473	XinxinApril2008methanolext	02April2008_815_1_10	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
474	XinxinApril2008methanolext	02April2008_815_2_7	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
475	XinxinApril2008methanolext	02April2008_815_2_10	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
476	XinxinApril2008methanolext	02April2008_815_3_7	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
477	XinxinApril2008methanolext	02April2008_815_3_10	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
478	XinxinApril2008methanolext	02April2008_815_4_7	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
479	XinxinApril2008methanolext	02April2008_815_4_10	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
480	XinxinApril2008methanolext	02April2008_815_5_7	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
481	XinxinApril2008methanolext	02April2008_815_5_10	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
482	XinxinApril2008methanolext	02April2008_815_6_7	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
483	XinxinApril2008methanolext	02April2008_815_6_10	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
484	XinxinApril2008methanolext	02April2008_815_1_8	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
485	XinxinApril2008methanolext	02April2008_815_1_11	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
486	XinxinApril2008methanolext	02April2008_815_2_8	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
487	XinxinApril2008methanolext	02April2008_815_2_11	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
488	8/05April2008methanolext	02April2008_815_3_8	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	

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489	XinxinApril2008methanolext	02April2008_815_3_11	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
490	XinxinApril2008methanolext	02April2008_815_4_8	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
491	XinxinApril2008methanolext	02April2008_815_4_11	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
492	XinxinApril2008methanolext	02April2008_815_5_8	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
493	XinxinApril2008methanolext	02April2008_815_5_11	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
494	XinxinApril2008methanolext	02April2008_815_6_8	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
495	XinxinApril2008methanolext	02April2008_815_6_11	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
496	XinxinApril2008methanolext	02April2008_815_1_9	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
497	XinxinApril2008methanolext	02April2008_815_1_12	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
498	XinxinApril2008methanolext	02April2008_815_2_9	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
499	XinxinApril2008methanolext	02April2008_815_2_12	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
500	XinxinApril2008methanolext	02April2008_815_3_9	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
501	XinxinApril2008methanolext	02April2008_815_3_12	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
502	XinxinApril2008methanolext	02April2008_815_4_9	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
503	XinxinApril2008methanolext	02April2008_815_4_12	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
504	XinxinApril2008methanolext	02April2008_815_5_9	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
505	XinxinApril2008methanolext	02April2008_815_5_12	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
506	XinxinApril2008methanolext	02April2008_815_6_9	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
507	XinxinApril2008methanolext	02April2008_815_6_12	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
508	XinxinApril2008methanolext	02April2008_815_3_14	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	
509	XinxinApril2008methanolext	02April2008_815_3_16	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	
510	XinxinApril2008methanolext	02April2008_815_3_18	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	
511	8/05April2008methanolext	02April2008_815_3_20	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	

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512	XinxinApril2008methanolext	02April2008_815_3_21	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	
513	XinxinApril2008methanolext	02April2008_815_3_22	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	
514	XinxinApril2008methanolext	02April2008_815_3_23	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	
515	XinxinApril2008methanolext	02April2008_815_3_24	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H EchA19	C18 with MeOH:IPA	N	N	Y	Y	
516	XinxinApril2008methanolext	02April2008_815_3_25	echA19	Y	N	Y	N	N	24h	Y	N	N	control	C18 with MeOH:IPA	N	N	Y	Y	
517	XinxinApril2008methanolext	02April2008_815_4_13	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	
518	XinxinApril2008methanolext	02April2008_815_4_15	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	
519	XinxinApril2008methanolext	02April2008_815_4_17	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	
520	XinxinApril2008methanolext	02April2008_815_4_19	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H Hsd	C18 with MeOH:IPA	N	N	Y	Y	
521	XinxinApril2008	14April2008_820_1_1	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
522	XinxinApril2008	14April2008_820_1_2	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
523	XinxinApril2008	14April2008_820_1_3	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
524	XinxinApril2008	14April2008_820_1_4	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
525	XinxinApril2008	14April2008_820_1_5	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
526	XinxinApril2008	14April2008_820_1_6	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
527	XinxinApril2008	14April2008_820_1_11	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
528	XinxinApril2008	14April2008_820_1_12	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
529	XinxinApril2008	14April2008_820_1_13	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
530	XinxinApril2008	14April2008_820_1_14	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
531	XinxinApril2008	14April2008_820_2_1	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
532	XinxinApril2008	14April2008_820_2_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
533	XinxinApril2008	14April2008_820_2_3	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
534	XinxinApril2008	14April2008_820_2_4	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
535	XinxinApril2008	14April2008_820_2_5	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
536	XinxinApril2008	14April2008_820_2_6	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
537	XinxinApril2008	14April2008_820_2_7	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	

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538	XinxinApril2008	14April2008_820_2_8	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
539	XinxinApril2008	14April2008_820_2_9	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
540	XinxinApril2008	14April2008_820_2_10	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
541	XinxinApril2008	14April2008_819_1_1	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
542	XinxinApril2008	14April2008_819_1_2	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
543	XinxinApril2008	14April2008_819_2_1	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
544	XinxinApril2008	14April2008_819_2_2	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
545	XinxinApril2008	14April2008_820_1_1b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
546	XinxinApril2008	14April2008_820_1_2b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
547	XinxinApril2008	14April2008_820_1_3b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
548	XinxinApril2008	14April2008_820_1_4b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
549	XinxinApril2008	14April2008_820_1_5b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
550	XinxinApril2008	14April2008_820_1_6b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
551	XinxinApril2008	14April2008_820_1_11b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
552	XinxinApril2008	14April2008_820_1_12b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
553	XinxinApril2008	14April2008_820_1_13b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
554	XinxinApril2008	14April2008_820_1_14b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
555	XinxinApril2008	14April2008_820_2_1b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
556	XinxinApril2008	14April2008_820_2_2b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
557	XinxinApril2008	14April2008_820_2_3b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
558	XinxinApril2008	14April2008_820_2_4b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
559	XinxinApril2008	14April2008_820_2_5b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
560	XinxinApril2008	14April2008_820_2_6b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol	C18 with MeOH:IPA	N	N	Y	Y	
561	XinxinApril2008	14April2008_820_2_7b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
562	XinxinApril2008	14April2008_820_2_8b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
563	XinxinApril2008	14April2008_820_2_9b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
564	XinxinApril2008	14April2008_820_2_10b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
565	XinxinApril2008	14April2008_819_1_1b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
566	XinxinApril2008	14April2008_819_1_2b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
567	XinxinApril2008	14April2008_819_2_1b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
568	XinxinApril2008	14April2008_819_2_2b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
569	XinxinApril2008	23April2008_819_1_2b	Rv	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
570	XinxinApril2008	23April2008_819_2_1b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
571	XinxinApril2008	23April2008_819_2_2b	fadA5	Y	N	Y	N	N	24h	Y	N	N	cholesterol FadA5	C18 with MeOH:IPA	N	N	Y	Y	
572	XinxinApril2008	23April2008_815_5_6b	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
573	XinxinApril2008	23April2008_815_5_6b_2	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
574	Xinxin24Apr2008	02April2008_815_1_1b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
575	Xinxin24Apr2008	02April2008_815_1_4b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
576	Xinxin24Apr2008	02April2008_815_2_1b	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
577	Xinxin24Apr2008	02April2008_815_2_4b	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
578	Xinxin24Apr2008	02April2008_815_3_1b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
579	Xinxin24Apr2008	02April2008_815_3_4b	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
580	Xinxin24Apr2008	02April2008_815_4_1b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	
581	Xinxin24Apr2008	02April2008_815_4_4b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	
582	Xinxin24Apr2008	02April2008_815_5_1b	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
583	Xinxin24Apr2008	02April2008_815_5_4b	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
584	Xinxin24Apr2008	02April2008_815_6_1b	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
585	Xinxin24Apr2008	02April2008_815_6_4b	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
586	Xinxin24Apr2008	02April2008_815_1_2b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
587	Xinxin24Apr2008	02April2008_815_1_5b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
588	Xinxin24Apr2008	02April2008_815_2_2b	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
589	Xinxin24Apr2008	02April2008_815_2_5b	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
590	Xinxin24Apr2008	02April2008_815_3_2b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
591	Xinxin24Apr2008	02April2008_815_3_5b	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
592	Xinxin24Apr2008	02April2008_815_4_2b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	
593	Xinxin24Apr2008	02April2008_815_4_5b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
594	Xinxin24Apr2008	02April2008_815_5_2b	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
595	Xinxin24Apr2008	02April2008_815_5_5b	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
596	Xinxin24Apr2008	02April2008_815_6_2b	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	
597	Xinxin24Apr2008	02April2008_815_6_5b	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	
598	Xinxin24Apr2008	02April2008_815_1_3b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
599	Xinxin24Apr2008	02April2008_815_1_6b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
600	Xinxin24Apr2008	02April2008_815_2_3b	CDC	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
601	Xinxin24Apr2008	02April2008_815_2_6b	CDC	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
602	Xinxin24Apr2008	02April2008_815_3_3b	echA19	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
603	Xinxin24Apr2008	02April2008_815_3_6b	echA19	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
604	Xinxin24Apr2008	02April2008_815_4_3b	hsd	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	N	Y	
605	Xinxin24Apr2008	02April2008_815_4_6b	hsd	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	N	Y	
606	Xinxin24Apr2008	02April2008_815_5_3b	fadE29	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
607	Xinxin24Apr2008	02April2008_815_5_6b	fadE29	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
608	Xinxin24Apr2008	02April2008_815_6_3b	kstR	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH:IPA	N	N	Y	Y	
609	Xinxin24Apr2008	02April2008_815_6_6b	kstR	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH:IPA	N	N	Y	Y	
610	Xinxin24Apr2008	02April2008_815_1_7b	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
611	Xinxin24Apr2008	02April2008_815_1_10b	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
612	Xinxin24Apr2008	02April2008_815_2_7b	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
613	Xinxin24Apr2008	02April2008_815_2_10b	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
614	Xinxin24Apr2008	02April2008_815_3_7b	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
615	Xinxin24Apr2008	02April2008_815_3_10b	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
616	Xinxin24Apr2008	02April2008_815_4_7b	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
617	Xinxin24Apr2008	02April2008_815_4_10b	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
618	Xinxin24Apr2008	02April2008_815_5_7b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
619	Xinxin24Apr2008	02April2008_815_5_10b	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
620	Xinxin24Apr2008	02April2008_815_6_7b	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
621	Xinxin24Apr2008	02April2008_815_6_10b	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
622	Xinxin24Apr2008	30April2008_815_1_8b	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
623	Xinxin24Apr2008	30April2008_815_1_11b	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
624	Xinxin24Apr2008	30April2008_815_2_8b	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
625	Xinxin24Apr2008	30April2008_815_2_11b	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
626	Xinxin24Apr2008	30April2008_815_3_8b	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
627	Xinxin24Apr2008	30April2008_815_3_11b	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
628	Xinxin24Apr2008	30April2008_815_4_8b	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
629	Xinxin24Apr2008	30April2008_815_4_11b	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
630	Xinxin24Apr2008	30April2008_815_5_8b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
631	Xinxin24Apr2008	30April2008_815_5_11b	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
632	Xinxin24Apr2008	30April2008_815_6_8b	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
633	Xinxin24Apr2008	30April2008_815_6_11b	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
634	Xinxin24Apr2008	30April2008_815_1_9b	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
635	Xinxin24Apr2008	30April2008_815_1_12b	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
636	Xinxin24Apr2008	30April2008_815_2_9b	CDC	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
637	Xinxin24Apr2008	30April2008_815_2_12b	CDC	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
638	Xinxin24Apr2008	30April2008_815_3_9b	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
639	Xinxin24Apr2008	30April2008_815_3_12b	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
640	Xinxin24Apr2008	30April2008_815_4_9b	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
641	Xinxin24Apr2008	30April2008_815_4_12b	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
642	Xinxin24Apr2008	30April2008_815_5_9b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
643	Xinxin24Apr2008	30April2008_815_5_12b	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
644	Xinxin24Apr2008	30April2008_815_6_9b	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
645	Xinxin24Apr2008	01May2008_815_5_7b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
646	Xinxin24Apr2008	01May2008_815_5_10b	fadE29	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
647	Xinxin24Apr2008	01May2008_815_6_11b	kstR	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
648	Xinxin24Apr2008	08May2008_815_1_7b	Rv	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
649	Xinxin24Apr2008	08May2008_815_1_10b	Rv	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	

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650	Xinxin24Apr2008	08May2008_815_3_7b	echA19	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
651	Xinxin24Apr2008	08May2008_815_3_10b	echA19	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	Y	Y	
652	Xinxin24Apr2008	08May2008_815_4_7b	hsd	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
653	Xinxin24Apr2008	08May2008_815_4_10b	hsd	Y	N	Y	N	N	24h	Y	N	N	pregnenolone	C18 with MeOH:IPA	N	N	N	Y	
654	Xinxin24Apr2008	08May2008_815_5_7b	fadE29	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	Y	Y	
655	Xinxin24Apr2008	08May2008_815_6_7b	kstR	Y	N	Y	N	N	24h	Y	N	N	dehydroepiandrosterone	C18 with MeOH:IPA	N	N	N	Y	
656	Xinxin24Apr2008	28April08_824_1	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH	N	N	N	Y	
657	Xinxin24Apr2008	28April08_824_2	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with MeOH	N	N	N	Y	
658	Xinxin24Apr2008	28April08_824_3	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH	N	N	N	Y	
659	Xinxin24Apr2008	28April08_824_4	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with MeOH	N	N	N	Y	
660	Xinxin24Apr2008	28April08_824_5	Rv	Y	N	Y	N	N	24h	Y	N	N	N	C18 with MeOH	N	N	N	Y	
661	Xinxin24Apr2008	28April08_824_1b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with IPA	N	N	N	Y	
662	Xinxin24Apr2008	28April08_824_2b	Rv	Y	N	Y	N	N	24h	Y	N	N	R:H	C18 with IPA	N	N	N	Y	
663	Xinxin24Apr2008	28April08_824_3b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with IPA	N	N	N	Y	
664	Xinxin24Apr2008	28April08_824_4b	Rv	Y	N	Y	N	N	24h	Y	N	N	T:H	C18 with IPA	N	N	N	Y	
665	Xinxin24Apr2008	28April08_824_5b	Rv	Y	N	Y	N	N	24h	Y	N	N	N	C18 with IPA	N	N	N	Y	
666	Xinxin24Apr2008	21May2008_839_1	Rv	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
667	Xinxin24Apr2008	21May2008_839_3	Rv	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
668	Xinxin24Apr2008	21May2008_839_1b	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
669	Xinxin24Apr2008	21May2008_839_3b	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
670	Xinxin24Apr2008	23May2008_839_2_2	Rv	Y	N	Y	N	N	24h	N	N	N	NA	CHCl3:MeOH	N	N	N	Y	
671	Xinxin24Apr2008	23May2008_839_4_2	Rv	Y	N	Y	N	N	24h	N	N	N	NA	CHCl3:MeOH	N	N	N	Y	
672	Xinxin24Apr2008	28May2008_839_1A	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
673	Xinxin24Apr2008	28May2008_839_1Ab	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
674	Xinxin24Apr2008	28May2008_839_3A	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
675	Xinxin24Apr2008	28May2008_839_3Ab	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:IPA	N	N	N	Y	
676	01June2008	01June2008_844_RVR_1	Rv	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
677	01June2008	01June2008_844_FadA5R_1	fadA5	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
678	01June2008	01June2008_844_ST146R_1	CDC	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
679	01June2008	01June2008_844_ST144R_1	hsd	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
680	01June2008	01June2008_844_ST152R_1	fadE29	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
681	01June2008	01June2008_844_ST154R_1	hsaA	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
682	01June2008	01June2008_844_RVT_1	Rv	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
683	01June2008	01June2008_844_FadA5T_1	fadA5	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
684	01June2008	01June2008_844_ST146T_1	CDC	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
685	01June2008	01June2008_844_ST144T_1	hsd	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
686	01June2008	01June2008_844_ST151T_1	fadD19	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
687	01June2008	01June2008_844_ST152T_1	fadE29	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
688	01June2008	01June2008_844_ST154T_1	hsaA	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
689	01June2008	01June2008_844_RVR_1b	Rv	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
690	01June2008	01June2008_844_FadA5R_1b	fadA5	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
691	01June2008	01June2008_844_ST146R_1b	CDC	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
692	01June2008	01June2008_844_ST144R_1b	hsd	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
693	01June2008	01June2008_844_ST152R_1b	fadE29	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
694	01June2008	01June2008_844_ST154R_1b	hsaA	Y	R	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
695	01June2008	01June2008_844_RVT_1b	Rv	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
696	01June2008	01June2008_844_FadA5T_1b	fadA5	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
697	01June2008	01June2008_844_ST146T_1b	CDC	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
698	01June2008	01June2008_844_ST144T_1b	hsd	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
699	01June2008	01June2008_844_ST151T_1b	fadD19	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
700	01June2008	01June2008_844_ST152T_1b	fadE29	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
701	01June2008	01June2008_844_ST154T_1b	hsaA	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with IPA	N	N	Y	Y	
702	01June2008	04June2008_844_RVR_CM	Rv	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
703	01June2008	04June2008_844_FadA5R_CM	fadA5	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
704	01June2008	04June2008_844_ST146R_CM	CDC	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
705	01June2008	04June2008_844_ST144R_CM	hsd	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
706	01June2008	04June2008_844_ST151R_CM	fadD19	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
707	01June2008	04June2008_844_ST152R_CM	fadE29	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
708	01June2008	04June2008_844_ST154R_CM	hsaA	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
709	01June2008	04June2008_844_RVT_CM	Rv	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
710	01June2008	04June2008_844_FadA5T_CM	fadA5	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
711	01June2008	05June2008_844_ST146T_CM	CDC	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
712	01June2008	05June2008_844_ST144T_CM	hsd	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
713	01June2008	05June2008_844_ST151T_CM	fadD19	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
714	01June2008	05June2008_844_ST152T_CM	fadE29	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
715	01June2008	05June2008_844_ST154T_CM	hsaA	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
716	01June2008	05June2008_844_RVR_T_1b	Rv	Y	T	Y	N	N	24h	N	Y	N	NA	C18 with MeOH:H2O	N	N	N	N	
717	01June2008	07June2008_844_RVR_CM	Rv	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
718	01June2008	07June2008_844_FadA5R_CM	fadA5	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
719	01June2008	07June2008_844_ST146R_CM	CDC	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
720	01June2008	07June2008_844_ST144R_CM	hsd	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
721	01June2008	07June2008_844_ST151R_CM	fadD19	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
722	01June2008	07June2008_844_ST152R_CM	fadE29	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
723	01June2008	07June2008_844_ST154R_CM	hsaA	Y	R	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
724	01June2008	07June2008_844_RVT_CM	Rv	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
725	01June2008	07June2008_844_FadA5T_CM	fadA5	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
726	01June2008	07June2008_844_ST146T_CM	CDC	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
727	01June2008	07June2008_844_ST144T_CM	hsd	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
728	01June2008	07June2008_844_ST151T_CM	fadD19	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
729	01June2008	07June2008_844_ST152T_CM	fadE29	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
730	01June2008	07June2008_844_ST154T_CM	hsaA	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
731	01June2008	16June2008_844_ST146T_CM_w	CDC	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
732	01June2008	16June2008_844_ST152T_CM_w	fadE29	Y	T	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
733	01June2008	17June2008_844_ST146T_PE in CM	CDC	Y	T	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N	

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				Cholesterol	Label	Tween-80	Tyloxypol													
734	01June2008	30June2008_852_RVA_PE	Rv	N	N	Y	N	N		24h	N	N	Y	NA	PE	N	N	N	N	
735	01June2008	30June2008_852_ST146A_PE	CDC	N	N	Y	N	N		24h	N	N	Y	NA	PE	N	N	N	N	
736	01June2008	30June2008_852_ST146D_PE	CDC	Y	N	Y	N	N		24h	N	N	Y	NA	PE	N	N	Y	Y	
737	01June2008	30June2008_852_ST152A_PE	fadE29	N	N	Y	N	N		24h	N	N	Y	NA	PE	N	N	N	N	
738	01June2008	30June2008_852_ST152D_PE	fadE29	Y	N	Y	N	N		24h	N	N	Y	NA	PE	N	N	N	N	
739	01June2008	30June2008_852_ST144A_PE	hsd	N	N	Y	N	N		24h	N	N	Y	NA	PE	N	N	N	N	
740	01June2008	30June2008_852_ST153D_PE	mmpL5	Y	N	Y	N	N		24h	N	N	Y	NA	PE	N	N	Y	Y	
741	01June2008	03July2008_852_RvA_CM	Rv	N	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
742	01June2008	03July2008_852_RvB_CM	Rv	Y	T:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
743	01June2008	03July2008_852_RvC_CM	Rv	Y	R:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
744	01June2008	03July2008_852_ST146A_CM	CDC	N	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
745	01June2008	03July2008_852_ST146B_CM	CDC	Y	T:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
746	01June2008	03July2008_852_ST146C_CM	CDC	Y	R:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
747	01June2008	03July2008_852_ST146D_CM	CDC	Y	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
748	01June2008	03July2008_852_ST76A_CM	fadA5	N	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
749	01June2008	03July2008_852_ST76B_CM	fadA5	Y	T:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
750	01June2008	03July2008_852_ST76C_CM	fadA5	Y	R:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
751	01June2008	03July2008_852_ST144A_CM	hsd	N	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
752	01June2008	03July2008_852_ST144B_CM	hsd	Y	T:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
753	01June2008	03July2008_852_ST144C_CM	hsd	Y	R:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
754	01June2008	03July2008_852_ST151A_CM	fadD19	N	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
755	01June2008	03July2008_852_ST151B_CM	fadD19	Y	T:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
756	01June2008	03July2008_852_ST151C_CM	fadD19	Y	R:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
757	01June2008	03July2008_852_ST152A_CM	fadE29	N	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
758	01June2008	03July2008_852_ST152B_CM	fadE29	Y	T:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
759	01June2008	03July2008_852_ST152C_CM	fadE29	Y	R:N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
760	01June2008	03July2008_852_ST152D_CM	fadE29	Y	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
761	01June2008	03July2008_852_ST153A_CM	mmpL5	N	N	Y	N	N		24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	

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				Cholesterol	Label	Tween-80	Tyloxypol													
762	01June2008	03July2008_852_ST153B_CM	mmpL5	Y	T:N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		
763	01June2008	03July2008_852_ST153C_CM	mmpL5	Y	R:N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		
764	01June2008	03July2008_852_ST153D_CM	mmpL5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		
765	Xinxin11Aug08	15Aug08_865_RvPE-1	Rv	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		
766	Xinxin11Aug08	22Aug08_865_RvPE	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		
767	Xinxin07Nov08	15Nov08_901_ST146c_PE	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		contaminated by detergents
768	Xinxin07Nov08	15Nov08_901_ST144c_PE	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		contaminated by detergents
769	Xinxin07Nov08	15Nov08_901_ST160c_PE	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		contaminated by detergents
770	Xinxin07Nov08	17Nov08_901_ST146c	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents
771	Xinxin07Nov08	17Nov08_901_ST144c	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y		contaminated by detergents
772	Xinxin07Nov08	17Nov08_901_ST160c	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents
773	Xinxin07Nov08	17Nov08_901_ST146D	CDC	N	N	Y	N	dehydroepiandrosterone	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		contaminated by detergents
774	Xinxin07Nov08	17Nov08_901_ST144D	hsd	N	N	Y	N	dehydroepiandrosterone	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		contaminated by detergents
775	Xinxin07Nov08	17Nov08_901_ST160D	hsd comp	N	N	Y	N	dehydroepiandrosterone	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		contaminated by detergents
776	Xinxin07Nov08	17Nov08_901_ST146T	CDC	N	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		contaminated by detergents
777	Xinxin07Nov08	17Nov08_901_ST144T	hsd	N	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		contaminated by detergents
778	Xinxin07Nov08	17Nov08_901_ST160T	hsd comp	N	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N		contaminated by detergents
779	Xinxin07Nov08	19Nov08_901_ST146c_washed	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents
780	Xinxin07Nov08	19Nov08_901_ST146c_washed_2	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents
781	Xinxin07Nov08	19Nov08_901_ST144c_washed	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y		contaminated by detergents
782	Xinxin07Nov08	19Nov08_901_ST160c_washed	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents
783	Xinxin07Nov08	20Nov08_901_ST146c_PE	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		contaminated by detergents
784	Xinxin07Nov08	20Nov08_901_ST144c_PE	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		contaminated by detergents
785	Xinxin07Nov08	20Nov08_901_ST160c_PE	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N		contaminated by detergents
786	Xinxin07Nov08	20Nov08_901_ST146c_washed	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents
787	Xinxin07Nov08	20Nov08_901_ST146c_washed_2	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents
788	Xinxin07Nov08	20Nov08_901_ST144c_washed	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y		contaminated by detergents
789	Xinxin07Nov08	20Nov08_901_ST160c_washed	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y		contaminated by detergents

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
790	Xinxin07Nov08	20Nov08_901_ST146c_PE_1	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N	contaminated by detergents
791	Xinxin07Nov08	20Nov08_901_ST144c_PE_1	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N	contaminated by detergents
792	Xinxin07Nov08	20Nov08_901_ST160c_PE_1	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	PE	N	N	N	N	contaminated by detergents
793	Xinxin07Nov08	20Nov08_901_ST146c_washed_1	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	contaminated by detergents
794	Xinxin07Nov08	20Nov08_901_ST144c_washed_1	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	contaminated by detergents
795	Xinxin07Nov08	20Nov08_901_ST160c_washed_1	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	contaminated by detergents
796	Xinxin07Nov08	27Nov08_901_ST146c_w	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
797	Xinxin07Nov08	27Nov08_901_ST144c_w	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
798	Xinxin07Nov08	27Nov08_901_ST160c_w	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	N	
799	Xinxin07Nov08	03Dec08_901_ST146_1	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
800	Xinxin07Nov08	03Dec08_901_ST146_2	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
801	Xinxin07Nov08	03Dec08_901_ST146_3	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
802	Xinxin07Nov08	03Dec08_901_ST146_7	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
803	Xinxin07Nov08	03Dec08_901_ST144_1	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
804	Xinxin07Nov08	03Dec08_901_ST144_2	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
805	Xinxin07Nov08	03Dec08_901_ST144_3	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
806	Xinxin07Nov08	03Dec08_901_ST144_7	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
807	Xinxin07Nov08	03Dec08_901_ST160_1	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
808	Xinxin07Nov08	03Dec08_901_ST160_2	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
809	Xinxin07Nov08	03Dec08_901_ST160_3	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
810	Xinxin07Nov08	03Dec08_901_ST160_7	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
811	Xinxin07Nov08	04Dec08_901_ST146_4	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
812	Xinxin07Nov08	04Dec08_901_ST146_5	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
813	Xinxin07Nov08	04Dec08_901_ST146_6	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
814	Xinxin07Nov08	04Dec08_901_ST144_4	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
815	Xinxin07Nov08	04Dec08_901_ST144_5	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
816	Xinxin07Nov08	04Dec08_901_ST144_6	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
817	Xinxin07Nov08	04Dec08_901_ST160_4	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
818	Xinxin07Nov08	04Dec08_901_ST160_5	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
819	Xinxin07Nov08	04Dec08_901_ST160_6	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
820	Xinxin07Nov08	09Dec08_905_ST146_1	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
821	Xinxin07Nov08	09Dec08_905_ST146_2	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
822	Xinxin07Nov08	09Dec08_905_ST146_3	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
823	Xinxin07Nov08	09Dec08_905_ST146_4	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
824	Xinxin07Nov08	09Dec08_905_ST146_5	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
825	Xinxin07Nov08	09Dec08_905_ST146_6	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
826	Xinxin07Nov08	09Dec08_905_ST144_1	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
827	Xinxin07Nov08	09Dec08_905_ST144_2	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
828	Xinxin07Nov08	09Dec08_905_ST144_3	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
829	Xinxin07Nov08	09Dec08_905_ST144_4	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
830	Xinxin07Nov08	09Dec08_905_ST144_5	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
831	Xinxin07Nov08	09Dec08_905_ST144_6	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
832	Xinxin07Nov08	09Dec08_905_ST160_1	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
833	Xinxin07Nov08	09Dec08_905_ST160_2	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
834	Xinxin07Nov08	09Dec08_905_ST160_3	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
835	Xinxin07Nov08	09Dec08_905_ST160_4	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
836	Xinxin07Nov08	09Dec08_905_ST160_5	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
837	Xinxin07Nov08	09Dec08_905_ST160_6	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
838	Xinxin07Nov08	12Dec08_905_ST146_1	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
839	Xinxin07Nov08	12Dec08_905_ST146_2	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
840	Xinxin07Nov08	12Dec08_905_ST146_3	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
841	Xinxin07Nov08	12Dec08_905_ST144_1	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
842	Xinxin07Nov08	12Dec08_905_ST144_2	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
843	Xinxin07Nov08	12Dec08_905_ST144_3	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
844	Xinxin07Nov08	12Dec08_905_ST160_1	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
845	Xinxin07Nov08	12Dec08_905_ST160_2	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions				Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
				Cholesterol	Label	Tween-80	Tyloxypol												
846	Xinxin07Nov08	12Dec08_905_ST160_3	hsd comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
847	Xinxin07Nov08	12Dec08_905_ST152_1	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
848	Xinxin07Nov08	12Dec08_905_ST152_2	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
849	Xinxin07Nov08	12Dec08_905_ST152_3	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
850	Xinxin07Nov08	12Dec08_905_ST46_C3	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
851	Xinxin07Nov08	12Dec08_905_ST46_C5	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
852	Xinxin07Nov08	12Dec08_905_ST46_C12	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
853	Xinxin07Nov08	12Dec08_905_ST46_C24	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
854	Xinxin07Nov08	12Dec08_905_ST46_C29	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
855	Xinxin07Nov08	12Dec08_905_ST46_C30	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
856	Xinxin07Nov08	12Dec08_905_ST46_C34	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
857	Xinxin07Nov08	16Dec08_915-5	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
858	Xinxin07Nov08	16Dec08_915-67	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
859	Xinxin07Nov08	16Dec08_915-97	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
860	Xinxin07Nov08	18Dec08_ST152_12_13	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
861	Xinxin07Nov08	19Dec08_913_5_6	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
862	Xinxin07Nov08	19Dec08_913_7	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
863	Xinxin07Nov08	19Dec08_913_8	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
864	Xinxin07Nov08	19Dec08_913_9	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
865	Xinxin07Nov08	19Dec08_913_11_15	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
866	Xinxin07Nov08	19Dec08_913_52_54	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
867	Xinxin07Nov08	19Dec08_913_71_75	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
868	Xinxin07Nov08	19Dec08_913_93_99	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
869	Xinxin07Nov08	19Dec08_910_3_10	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
870	Xinxin07Nov08	19Dec08_910_11_17	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
871	Xinxin07Nov08	19Dec08_910_23	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
872	Xinxin07Nov08	19Dec08_910_24_28	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
873	Xinxin07Nov08	19Dec08_910_38_72	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
874	Xinxin07Nov08	19Dec08_910_73_151	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
875	Xinxin07Nov08	19Dec08_910_152e	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
876	Xinxin07Nov08	19Dec08_916_5	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
877	Xinxin07Nov08	19Dec08_916_7	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
878	Xinxin07Nov08	19Dec08_916_20	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
879	Xinxin07Nov08	19Dec08_916_40	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
880	Xinxin07Nov08	19Dec08_916_43	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
881	Xinxin07Nov08	19Dec08_916_49	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
882	Xinxin07Nov08	19Dec08_916_53	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
883	Xinxin07Nov08	19Dec08_916_57	hsd	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	Y	
884	Xinxin07Nov08	19Dec08_918_3_5	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
885	Xinxin07Nov08	19Dec08_918_9	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
886	Xinxin07Nov08	19Dec08_918_11	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
887	Xinxin07Nov08	19Dec08_918_12_13	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	N	N	
888	Xinxin07Nov08	19Dec08_918_15	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
889	Xinxin07Nov08	19Dec08_918_17	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
890	Xinxin07Nov08	19Dec08_918_29	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
891	Xinxin07Nov08	19Dec08_918_39	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
892	Xinxin07Nov08	19Dec08_918_43_45	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
893	Xinxin07Nov08	19Dec08_918_51	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
894	Xinxin07Nov08	19Dec08_918_59_61	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH, silica column	N	N	Y	Y	
895	Xinxin07Nov08	22Dec08_905_ST146_C14	CDC	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
896	Xinxin07Nov08	22Dec08_905_ST152_C14	fadE29	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
897	Xinxin07Nov08	22Dec08_905_ST144_C14	hsd	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	
898	Xinxin07Nov08	22Dec08_905_ST160_C14	hsd comp	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
899	Xinxin07Nov08	24Dec08_905_ST146_C14	CDC	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
900	Xinxin07Nov08	24Dec08_852_RvB_PE	Rv	Y	T:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
901	Xinxin07Nov08	24Dec08_852_RvC_PE	Rv	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
902	Xinxin07Nov08	24Dec08_852_ST76B_PE	fadA5	Y	T:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
903	Xinxin07Nov08	24Dec08_852_ST76C_PE	fadA5	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
904	Xinxin07Nov08	24Dec08_852_ST146B_PE	CDC	Y	T:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
905	Xinxin07Nov08	24Dec08_852_ST146C_PE	CDC	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
906	Xinxin07Nov08	24Dec08_852_ST144B_PE	hsd	Y	T:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
907	Xinxin07Nov08	24Dec08_852_ST144C_PE	hsd	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
908	Xinxin07Nov08	24Dec08_852_ST151B_PE	fadD19	Y	T:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
909	Xinxin07Nov08	24Dec08_852_ST151C_PE	fadD19	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
910	Xinxin07Nov08	24Dec08_852_ST152B_PE	fadE29	Y	T:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
911	Xinxin07Nov08	24Dec08_852_ST152C_PE	fadE29	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
912	Xinxin07Nov08	24Dec08_852_ST153B_PE	mmpL5	Y	T:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
913	Xinxin07Nov08	24Dec08_852_ST153C_PE	mmpL5	Y	R:N	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
914	Xinxin07Nov08	24Dec08_852_ST76T_PE	fadA5	Y	T	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
915	Xinxin07Nov08	24Dec08_852_ST144T_PE	hsd	Y	T	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
916	Xinxin07Nov08	24Dec08_852_ST151T_PE	fadD19	Y	T	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
917	Xinxin07Nov08	24Dec08_852_ST152T_PE	fadE29	Y	T	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
918	Xinxin07Nov08	24Dec08_852_ST154T_PE	mmpL5	Y	T	Y	N	N	24h	N	N	Y	NA	PE	N	N	Y	Y	
919	Xinxin07Nov08	29Jan09_927_CDC30min_C14	CDC	Y	C14	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
920	Xinxin07Nov08	29Jan09_927_CDC1h_C14	CDC	Y	C14	Y	N	N	1h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
921	Xinxin07Nov08	29Jan09_927_CDC2h_C14	CDC	Y	C14	Y	N	N	2h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
922	Xinxin07Nov08	29Jan09_927_CDCON_C14	CDC	Y	C14	Y	N	N	14h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
923	Xinxin07Nov08	30Jan09_927_Rv_4C14	Rv	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
924	Xinxin07Nov08	30Jan09_927_hsaA_4C14	hsaA	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
925	Xinxin07Nov08	30Jan09_927_fadA5_4C14	fadA5	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
926	Xinxin07Nov08	30Jan09_ST146_26C14	CDC	Y	26-C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
927	Xinxin07Nov08	30Jan09_ST144_26C14	hsd	Y	26-C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	
928	Xinxin07Nov08	30Jan09_ST160_26C14	hsd comp	Y	26-C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
929	Xinxin07Nov08	30Jan09_ST152_26C14	fadE29	Y	26-C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions					Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
				Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates											
930	Xinxin07Nov08	03Feb09_927_CDC	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
931	Xinxin07Nov08	03Feb09_927_fadA5	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
932	Xinxin07Nov08	03Feb09_927_Rv	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
933	Xinxin07Nov08	03Feb09_927_hsaA	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
934	Xinxin07Nov08	05Feb09_927_fadA5	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
935	Xinxin07Nov08	05Feb09_927_hsaA	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
936	Xinxin07Nov08	05Feb09_927_CDC	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
937	Xinxin07Nov08	05Feb09_927_Rv	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
938	Xinxin07Nov08	06Feb09_927_fadA5	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
939	Xinxin07Nov08	06Feb09_927_hsaA	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
940	Xinxin07Nov08	06Feb09_927_Rv	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
941	Xinxin07Nov08	06Feb09_927_CDC	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
942	Xinxin07Nov08	11Feb09_927_CDC	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
943	Xinxin07Nov08	11Feb09_927_fadA5	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
944	Xinxin07Nov08	11Feb09_927_Rv	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
945	Xinxin07Nov08	11Feb09_927_hsaA	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
946	Xinxin07Nov08	13Feb09_927_hsaA_1	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
947	Xinxin07Nov08	13Feb09_927_hsaA_2	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
948	Xinxin07Nov08	13Feb09_927_hsaA_3	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
949	Xinxin07Nov08	13Feb09_927_hsaA_1_1	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
950	Xinxin07Nov08	13Feb09_927_hsaA_2_1	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
951	Xinxin07Nov08	13Feb09_927_hsaA_3_1	hsaA	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
952	Xinxin04March09	04Mar09_927_fadA5_1	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	
953	Xinxin04March09	04Mar09_927_fadA5_2	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	
954	Xinxin04March09	04Mar09_927_fadA5_3	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
955	Xinxin04March09	04Mar09_927_fadA5_4	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
956	Xinxin04March09	05Mar09_927_fadA5_3_7min	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	N	
957	Xinxin04March09	06Mar09_949_Rv_1_C14	Rv	Y	C14	Y	N	N	1h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Time point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
958	Xinxin04March09	06Mar09_949_Rv_1_C14_1	Rv	Y	C14	Y	N	N	1h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
959	Xinxin04March09	06Mar09_949_Rv_5_C14	Rv	Y	C14	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
960	Xinxin04March09	06Mar09_949_Rv_24_C14	Rv	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
961	Xinxin04March09	06Mar09_949_Rv_48_C14	Rv	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
962	Xinxin04March09	08Mar09_949_FadA5_1_C14	fadA5	Y	C14	Y	N	N	1h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
963	Xinxin04March09	08Mar09_949_FadA5_5_C14	fadA5	Y	C14	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
964	Xinxin04March09	08Mar09_949_FadA5_24_C14	fadA5	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
965	Xinxin04March09	08Mar09_949_FadA5_48_C14	fadA5	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
966	Xinxin04March09	08Mar09_949_ST93_1_C14	fadA5 comp	Y	C14	Y	N	N	1h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
967	Xinxin04March09	08Mar09_949_ST93_5_C14	fadA5 comp	Y	C14	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
968	Xinxin04March09	08Mar09_949_ST93_24_C14	fadA5 comp	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
969	Xinxin04March09	08Mar09_949_ST93_48_C14	fadA5 comp	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
970	Xinxin04March09	11Mar09_949_Rv_1_C14_1	Rv	Y	C14	Y	N	N	1h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
971	Xinxin04March09	19Mar09_949_Rv_48filtrate_EA	Rv	Y	C14	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	Y	
972	Xinxin04March09	20Mar09_949_Rv_48filtrate_EA_1	Rv	Y	C14	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	Y	
973	Xinxin04March09	20Mar09_949_Rv_48filtrate_EA and AD	Rv	Y	C14	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	Y	
974	Xinxin04March09	20Mar09_949_Rv_48filtrate_EA and AD_2	Rv	Y	C14	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	Y	
975	Xinxin04March09	20Mar09_949_Rv_5_C14	Rv	Y	C14	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
976	Xinxin04March09	20Mar09_949_Rv_24_C14	Rv	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
977	Xinxin04March09	20Mar09_949_Rv_48_C14	Rv	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
978	Xinxin04March09	20Mar09_949_FadA5_24_C14	fadA5	Y	C14	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
979	Xinxin04March09	20Mar09_949_ST93_1_C14	fadA5 comp	Y	C14	Y	N	N	1h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
980	Xinxin04March09	20Mar09_949_ST93_48_C14	fadA5 comp	Y	C14	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
981	Xinxin04March09	20Mar09_949_Rv_1_fil_EA	Rv	Y	C14	Y	N	N	1h	N	Y	N	NA	EA	N	Y	N	Y	
982	Xinxin04March09	20Mar09_949_FadA5_1_fil_EA	fadA5	Y	C14	Y	N	N	1h	N	Y	N	NA	EA	N	N	N	Y	
983	Xinxin04March09	20Mar09_949_ST93_1_fil_EA	fadA5 comp	Y	C14	Y	N	N	1h	N	Y	N	NA	EA	N	Y	N	Y	
984	Xinxin04March09	20Mar09_949_Rv_48_fil_EA	Rv	Y	C14	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
985	Xinxin04March09	20Mar09_949_FadA5_48_fil_EA	fadA5	Y	C14	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	Y	
986	Xinxin04March09	20Mar09_949_ST93_48_fil_EA	fadA5comp	Y	C14	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	Y	
987	Xinxin04March09	25Mar09_949_Rv_5h	Rv	Y	N	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
988	Xinxin04March09	25Mar09_949_Rv_48h	Rv	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	N	Y	
989	Xinxin04March09	26Mar09_949_fadA5_Filtrate_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
990	Xinxin04March09	26Mar09_949_fadA5_Filtrate_EA_2	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
991	Xinxin04March09	26Mar09_949_Rv_Filtrate_EA_2	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
992	Xinxin04March09	27Mar09_949_ST93_Filtrate_EA	fadA5comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
993	Xinxin04March09	27Mar09_949_ST93_Filtrate_EA_2	fadA5comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
994	Xinxin04March09	27Mar09_949_ST93_Filtrate_EA_3	fadA5comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
995	Xinxin04March09	27Mar09_949_FadA5_Filtrate_EA_3	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	m/z 277,309, 295
996	Xinxin04March09	27Mar09_949_Rv_Filtrate_EA_3	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
997	Xinxin04March09	27Mar09_949_ST93_Filtrate_EA_4	fadA5comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
998	Xinxin04March09	27Mar09_949_FadA5_Filtrate_EA_4	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
999	Xinxin04March09	27Mar09_949_Rv_Filtrate_EA_4	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1000	Xinxin04March09	31Mar09_949_Rv_48h_F_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1001	Xinxin04March09	31Mar09_949_fadA5_48h_F_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	m/z 277,309, 295
1002	Xinxin04March09	31Mar09_949_ST93_48h_F_EA	fadA5comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1003	Xinxin04March09	01April09_949_Rv_F_AC_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1004	Xinxin04March09	01April09_949_FadA5_F_AC_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
1005	Xinxin04March09	01April09_949_ST93_F_AC_EA	fadA5comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1006	Xinxin04March09	03April09_949_Rv_F_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1007	Xinxin04March09	03April09_949_FadA5_F_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
1008	Xinxin04March09	03April09_949_ST93_F_EA	fadA5comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1009	Xinxin04March09	03April09_949_FadA5_F_EA_2	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
1010	Xinxin04March09	03April09_949_Rv_F_EA_2	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1011	Xinxin04March09	07April09_949_ST93_CM	fadA5comp	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1012	Xinxin04March09	07April09_949_FadA5_CM	fadA5	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	

				Growth conditions															
#	Project name	File name	Mutants	Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
1013	Xinxin04March09	07April09_949_Rv_CM	Rv	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1014	Xinxin04March09	07April09_949_Rv_CM_1	Rv	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1015	Xinxin04March09	13April09_949_ST93_CM	fadA5 comp	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1016	Xinxin04March09	13April09_949_FadA5_CM	fadA5	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1017	Xinxin04March09	13April09_949_Rv_CM	Rv	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1018	Xinxin04March09	15April09_984_FadA5_R_F_EA	fadA5	Y	R	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	N	m/z 277,309, 295
1019	Xinxin04March09	15April09_984_FadA5_T_F_EA	fadA5	Y	T	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	N	m/z 278,310, 296
1020	Xinxin04March09	15April09_984_FadA5_F_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	N	m/z 277,309, 295
1021	Xinxin04March09	15April09_984_Rv_1_F_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	N	
1022	Xinxin04March09	15April09_984_Rv_2_F_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	N	
1023	Xinxin04March09	15April09_984_ST93_F_EA	fadA5 comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	N	N	
1024	Xinxin04March09	16April09_984_FadA5_R_F_EA_1	fadA5	Y	R	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	N	m/z 277,309, 295
1025	Xinxin04March09	16April09_984_FadA5_T_F_EA_1	fadA5	Y	T	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	N	m/z 278,310, 296
1026	Xinxin04March09	16April09_984_FadA5_F_EA_1	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	N	m/z 277,309, 295
1027	Xinxin04March09	17April09_984_FadA5_R_CM	fadA5	Y	R	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1028	Xinxin04March09	17April09_984_FadA5_T_CM	fadA5	Y	T	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1029	Xinxin04March09	17April09_984_FadA5_1_CM	fadA5	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1030	Xinxin04March09	17April09_984_FadA5_R_CM_1	fadA5	Y	R	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1031	Xinxin04March09	17April09_984_FadA5_T_CM_1	fadA5	Y	T	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1032	Xinxin04March09	17April09_984_FadA5_1_CM_1	fadA5	Y	R	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1033	Xinxin04March09	17April09_984_Rv_1_CM_1	Rv	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1034	Xinxin04March09	17April09_984_ST93_1_CM_1	fadA5 comp	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1035	Xinxin04March09	20April09_986_CDC_F_EA	CDC	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	Y(trace)	
1036	Xinxin04March09	20April09_986_HSD_F_EA	had	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	Y(trace)	
1037	Xinxin04March09	21April09_986_CDC_F_AC_EA	CDC	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	Y(trace)	
1038	Xinxin04March09	21April09_986_ST93_F_EA	hsd comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	Y(trace)	
1039	Xinxin04March09	21April09_984_Rv_1_CM_1	Rv	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1040	Xinxin04March09	21April09_984_ST93_1_CM_1	fadA5 comp	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
1041	Xinxin04March09	22April09_984_HSD comp_F_EA	hsd comp	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	N	Y(trace)	
1042	Xinxin04March09	29April09_986_ST146_CM	CDC	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y(trace)	
1043	Xinxin04March09	29April09_986_ST144_CM	hsd	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y(trace)	
1044	Xinxin04March09	29April09_986_ST160_CM	hsd comp	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y(trace)	
1045	Xinxin04March09	29April09_986_ST146_F_EA	CDC	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	N	N	N	Y(trace)	
1046	Xinxin04March09	29April09_986_ST144_F_EA	hsd	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	N	N	N	Y(trace)	
1047	Xinxin04March09	29April09_986_ST160_F_EA	hsd comp	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	N	N	N	Y(trace)	
1048	Xinxin04March09	29April09_984_Rv_1_F_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	Y	N	Y	Y	
1049	Xinxin04March09	29April09_984_FadA5_1_F_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	Y	N	Y	Y	
1050	Xinxin04March09	29April09_984_ST93_1_F_EA	fadA5 comp	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	Y	N	Y	Y	
1051	Xinxin04March09	29April09_984_Rv_2_F_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	Y	N	Y	Y	
1052	Xinxin04March09	29April09_984_FadA5_2_F_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	Y	N	Y	Y	
1053	Xinxin04March09	29April09_984_ST93_2_F_EA	fadA5 comp	Y	N	Y	N	N	48h	N	Y	N	NA	CHCl3:MeOH	Y	N	Y	Y	
1054	Xinxin04March09	01May09_984_Rv_1_CM	Rv	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1055	Xinxin04March09	01May09_984_FadA5_1_CM	fadA5	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1056	Xinxin04March09	01May09_984_ST93_1_CM	fadA5 comp	Y	N	Y	N	N	48h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1057	Xinxin04March09	14May09_990_CDC_5_F_EA	CDC	Y	N	Y	N	N	5h	N	Y	N	NA	EA	N	N	Y	Y	
1058	Xinxin04March09	14May09_990_CDC_24_F_EA	CDC	Y	N	Y	N	N	24h	N	Y	N	NA	EA	N	N	Y	Y	
1059	Xinxin04March09	14May09_990_FadE29_5_F_EA	fadE29	Y	N	Y	N	N	5h	N	Y	N	NA	EA	N	N	Y	Y	
1060	Xinxin04March09	14May09_990_FadE29_24_F_EA	fadE29	Y	N	Y	N	N	24h	N	Y	N	NA	EA	N	N	Y	Y	
1061	Xinxin04March09	15May09_990_CDC_5_F_EA	CDC	Y	N	Y	N	N	5h	N	Y	N	NA	EA	N	N	Y	Y	
1062	Xinxin04March09	15May09_990_CDC_24_F_EA	CDC	Y	N	Y	N	N	24h	N	Y	N	NA	EA	N	N	Y	Y	
1063	Xinxin04March09	15May09_990_CDC_5_CM	CDC	Y	N	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1064	Xinxin04March09	15May09_990_CDC_24_CM	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1065	Xinxin04March09	15May09_990_FadE29_5_CM	fadE29	Y	N	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1066	Xinxin04March09	15May09_990_FadE29_24_CM	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1067	Xinxin04March09	15May09_986_Rv_3_F_EA	Rv	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	Y	Y	Y	
1068	Xinxin04March09	15May09_986_FadA5_3_F_EA	fadA5	Y	N	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
1069	Xinxin04March09	20May09_990_CDC_5_CM	CDC	Y	N	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1070	Xinxin04March09	20May09_990_CDC_24_CM	CDC	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1071	Xinxin04March09	20May09_990_FadE29_5_CM	fadE29	Y	N	Y	N	N	5h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1072	Xinxin04March09	20May09_990_FadE29_24_CM	fadE29	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	
1073	Xinxin04March09	20May09_984_FadA5_R_F_EA	fadA5	Y	R	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
1074	Xinxin04March09	20May09_984_FadA5_T_F_EA	fadA5	Y	T	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 278,310, 296
1075	Xinxin04March09	20May09_984_FadA5_R_F_EA_2	fadA5	Y	R	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 277,309, 295
1076	Xinxin04March09	20May09_984_FadA5_T_F_EA_2	fadA5	Y	T	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 278,310, 296
1077	Xinxin04March09	20May09_984_FadA5_R_F_EA_neg	fadA5	Y	R	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 275,307, 293
1078	Xinxin04March09	20May09_984_FadA5_T_F_EA_neg	fadA5	Y	T	Y	N	N	48h	N	Y	N	NA	EA	N	N	Y	Y	m/z 276,308, 294
1079	Xinxin24June09	24Jun09_1K_ST144_J9_EA	hsd	Y	N	Y	N	N	14h	N	Y	N	NA	EA	N	N	N	N	
1080	Xinxin24June09	24Jun09_1K_ST146_J9_EA	CDC	Y	N	Y	N	N	14h	N	Y	N	NA	EA	N	N	N	N	
1081	Xinxin24June09	24Jun09_1K_ST154_J9_EA	hsaA	Y	N	Y	N	N	14h	N	Y	N	NA	EA	N	N	Y	Y	
1082	Xinxin24June09	26Jun09_1K_ST146_30_EA	CDC	Y	C14	Y	N	N	30min	N	Y	N	NA	EA	N	N	N	Y	
1083	Xinxin24June09	26Jun09_1K_ST144_30_EA	hsd	Y	C14	Y	N	N	30min	N	Y	N	NA	EA	N	N	N	Y	
1084	Xinxin24June09	26Jun09_1K_ST154_30_EA	hsaA	Y	C14	Y	N	N	30min	N	Y	N	NA	EA	N	N	N	Y	
1085	Xinxin24June09	26Jun09_1K_ST146_24h_EA	CDC	Y	C14	Y	N	N	24h	N	Y	N	NA	EA	N	N	N	Y	
1086	Xinxin24June09	26Jun09_1K_ST144_24h_EA	hsd	Y	C14	Y	N	N	24h	N	Y	N	NA	EA	N	N	N	Y	
1087	Xinxin24June09	26Jun09_1K_ST154_24h_EA	hsaA	Y	C14	Y	N	N	24h	N	Y	N	NA	EA	N	N	N	Y	
1088	Xinxin24June09	07July09_1003_Rv_DP_EA	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EA	N	N	Y	Y	
1089	Xinxin24June09	07July09_1003_fadA5_DP_EA	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EA	N	N	Y	Y	
1090	Xinxin24June09	08July09_1K_Rv_DP_EA	Rv	Y	C14	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EA	N	N	Y	Y	
1091	Xinxin24June09	08July09_1K_fadA5_DP_EA	fadA5	Y	C14	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EA	N	N	Y	Y	
1092	Xinxin24June09	08July09_1K5_CDC_30min_EA	CDC	Y	N	Y	N	N	30min	N	Y	N	NA	EA	N	N	N	Y	
1093	Xinxin24June09	08July09_1K5_hsd_30min_EA	hsd	Y	N	Y	N	N	30min	N	Y	N	NA	EA	N	N	N	Y	
1094	Xinxin24June09	08July09_1K5_ST154_30min_EA	hsaA	Y	N	Y	N	N	30min	N	Y	N	NA	EA	N	N	N	Y	
1095	Xinxin24June09	10July09_1K3_Rv_DP_CM	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1096	Xinxin24June09	10July09_1K3_fadA5_DP_CM	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
1097	Xinxin24June09	10July09_1K5_CDC_30min_CM	CDC	Y	N	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	m/z 367,401
1098	Xinxin24June09	10July09_1K5_HSD_30min_CM	hsd	Y	N	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	m/z 367,401
1099	Xinxin24June09	10July09_1K5_hsaA_30min_CM	hsaA	Y	N	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	m/z 367,401
1100	Xinxin24June09	11July09_1K3_Rv_DP_CM	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1101	Xinxin24June09	11July09_1K3_fadA5_DP_CM	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1102	Xinxin24June09	11July09_1K5_CDC_30min_CM	CDC	Y	N	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	m/z 367,401
1103	Xinxin24June09	11July09_1K5_HSD_30min_CM	hsd	Y	N	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	m/z 367,401
1104	Xinxin24June09	11July09_1K5_hsaA_30min_CM	hsaA	Y	N	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	m/z 367,401
1105	Xinxin24June09	11July09_1K3_Rv_DP_CM_pos	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1106	Xinxin24June09	12July09_1K3_Rv_DP_CM_neg	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1107	Xinxin24June09	12July09_1K3_fadA5_DP_CM_neg	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1108	Xinxin24June09	12July09_1K3_Rv_DP_CM_pos	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1109	Xinxin24June09	12July09_1K3_fadA5_DP_CM_pos	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1110	Xinxin24June09	15July09_1K3_Rv_CM_pos	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1111	Xinxin24June09	15July09_1K3_fadA5_CM_pos	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1112	Xinxin24June09	15July09_1K_fadA5smeg_CM_pos	fadA5 smeg	Y	N	Y	N	N	30min	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	m/z 367,401
1113	Xinxin24June09	15July09_1K3_Rv_CM_neg	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1114	Xinxin24June09	15July09_1K3_fadA5_CM_neg	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	Y	Y	m/z 367,401
1115	Xinxin24June09	15July09_1K_fadA5smeg_CM_neg	fadA5 smeg	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	N	N	N	Y	
1116	Xinxin24June09	10Sep09_1k9_Rv_DP_EA	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EtOAc	N	Y	Y	Y	
1117	Xinxin24June09	10Sep09_1k9_fadA5_DP_EA	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EtOAc	N	N	Y	Y	
1118	Xinxin24June09	10Sep09_1k9_Rv_EA	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	EtOAc	N	Y	Y	Y	
1119	Xinxin24June09	10Sep09_1k9_fadA5_EA	fadA5	Y	N	Y	N	N	24h	N	Y	N	NA	EtOAc	N	N	Y	Y	
1120	Xinxin24June09	10Sep09_1k9_Rv_DP_EA1	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EtOAc	N	Y	Y	Y	
1121	Xinxin24June09	10Sep09_1k9_fadA5_DP_EA1	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EtOAc	N	N	Y	Y	
1122	Xinxin24June09	10Sep09_1k9_Rv_EA1	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	EtOAc	N	Y	Y	Y	
1123	Xinxin24June09	10Sep09_1k9_fadA5_EA1	fadA5	Y	N	Y	N	N	24h	N	Y	N	NA	EtOAc	N	N	Y	Y	
1124	Xinxin24June09	10Sep09_1k9_fadA5com_DP_EA	fadA5 comp	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EtOAc	N	Y	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
1125	Xinxin24June09	10Sep09_1k9_fadA5com_EA	fadA5 comp	Y	N	Y	N	N	24h	N	Y	N	NA	EtOAc	N	Y	Y	Y	
1126	Xinxin24June09	11Sep09_1k9_Rv_DP_CM	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1127	Xinxin24June09	11Sep09_1k9_Rv_CM	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1128	Xinxin24June09	11Sep09_1k9_fadA5_DP_CM	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1129	Xinxin24June09	11Sep09_1k9_fadA5_CM	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1130	Xinxin24June09	11Sep09_1k9_fadA5comp_DP_CM	fadA5 comp	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1131	Xinxin24June09	11Sep09_1k9_fadA5comp_CM	fadA5 comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1132	Xinxin24June09	11Sep09_waxy ester after 1 month	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1133	Xinxin24June09	01Oct09_1k9_Rv_DP_CM	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	Y	Y	Y	Y	
1134	Xinxin24June09	01Oct09_1k9_Rv_CM	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	Y	Y	Y	
1135	Xinxin24June09	01Oct09_1k9_fadA5_DP_CM	fadA5	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1136	Xinxin24June09	01Oct09_1k9_fadA5_CM	fadA5	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	N	Y	Y	
1137	Xinxin24June09	01Oct09_1k9_fadA5comp_DP_CM	fadA5 comp	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH	Y	Y	Y	Y	
1138	Xinxin24June09	01Oct09_1k9_fadA5comp_CM	fadA5 comp	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH	Y	Y	Y	Y	
1139	Xinxin24June09	14Oct09_waxy ester_HD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1140	Xinxin24June09	16Oct09_waxy ester_HD_1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1141	Xinxin24June09	16Oct09_waxy ester_HD_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1142	Xinxin24June09	27Oct09_1012_1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1143	Xinxin24June09	27Oct09_1012_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1144	Xinxin24June09	27Oct09_1012_top	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1145	Xinxin24June09	16Nov09_1014_4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1146	Xinxin24June09	16Nov09_1014_4_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1147	Xinxin24June09	16Nov09_1014_4_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Y	N	N	N	
1148	Xinxin24June09	26Nov09_1016_Rv_EA	Rv	Y	N	Y	N	N	24h	N	Y	N	NA	EtOAc	Y	Y	Y	N	
1149	Xinxin24June09	26Nov09_1016_RvDP_EA	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	Y	N	NA	EtOAc	Y	Y	N	N	
1150	Xinxin24June09	26Nov09_1016_RvCCl_EA	Rv	Y	N	Y	N	cholesteryl chloride	24h	N	Y	N	NA	EtOAc	Y	Y	N	N	
1151	Xinxin24June09	26Nov09_1016_Rv_CM	Rv	Y	N	Y	N	N	24h	N	N	Y	NA	CHCl3:MeOH		N	Y	Y	
1152	Xinxin24June09	26Nov09_1016_RvDP_CM	Rv	Y	N	Y	N	2,2'-dipyridyl	24h	N	N	Y	NA	CHCl3:MeOH		N	Y	Y	

#	Project name	File name	Mutants	Growth conditions: Cholesterol	Label	Tween-80	Tyloxypol	Inhibitor or other substrates	Tme point	Lysate sup	Filtrate sup	Cells	Incubation conditions[a]	Extraction[b]	Cholesterol-derived aploar lipid[c]	AD/ADD[d]	Cholest-4-en-3-one	Cholesterol	Other metabolites
1153	Xinxin24June09	26Nov09_1016_RvCCl_CM	Rv	Y	N	Y	N	cholesteryl chloride	24h	N	N	Y	NA	CHCl3:MeOH		N	N	N	

[a]Lysates were incubated with 150 µM sterol listed and 2.8 mM NAD^+, with or without ATP and coenzyme A. Conditions: 100 mM TAPS hydrochloride buffer, 150 mM NaCl, 30 mM $MgCl_2$, pH 8.5 or pH8.0, 30 °C. [b]Extraction methods are described in Chapter 4. [C] The cholesterol-derived apolar lipid as described in Chapter 4. [d]Androst-4-ene-3,17-dione and androsta-1,4-diene-3,17-dione.