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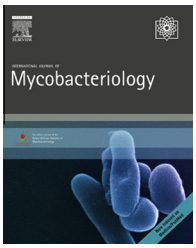

Short Communication

Use of an adipocyte model to study the transcriptional adaptation of *Mycobacterium tuberculosis* to store and degrade host fat

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ABSTRACT

During its persistence in the infected host, *Mycobacterium tuberculosis* (*Mtb*) accumulates host-derived fatty acids in intracytoplasmic lipid inclusions as triacylglycerols which serve primarily as carbon and energy reserves. The *Mtb* genome codes for more than 15 triacylglycerol synthases, 24 lipase/esterases, and seven cutinase-like proteins. Hence, we looked at the expression of the corresponding genes in intracellular bacilli persisting amidst the host triacylglycerols. We used the Mtb infected murine adipocyte model to ensure persistence and transcripts were quantified using real-time reverse transcriptase polymerase chain reaction. Dormancy and glyoxylate metabolism was confirmed by the upregulated expression of dosR and icl, respectively, by intra-adipocyte bacilli compared with in vitro growing bacilli. The study revealed that tgs1, tgs2, Rv3371, and mycolyltransferase Ag85A are the predominant triacylglycerol synthases, while lipF, lipH, lipJ, lipK, lipN, lipV, lipX, lipY, culp5, culp7, and culp6 are the predominant lipases/esterases used by *Mtb* for the storage and degradation of host-derived fat. Moreover, it was observed that many of these enzymes are used by *Mtb* during active replication rather than during nonreplicating persistence, indicating their probable function in cell wall synthesis.

Introduction

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, is one of the most successful pathogens in humans, killing 1.5 million people, including 360,000 human immunodeficiency virus-positive people in 2013 [1]. Eradication of this infectious killer is hampered by its ability to persist as dormant forms in the host despite a functional immune system and completion of the standard antitubercular regimen [2]. Antitubercular drugs in the standard regimen target the actively dividing population of the bacilli. Hence, the current antitubercular drug discovery programs aim at finding molecules that can target replicating as well as nonreplicating populations. Understanding what helps *Mtb* to persist as a

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metabolically dormant population in the host will help identify better target proteins and pathways for drug discovery programs.

In the host, *Mtb* is thought to depend on host-derived fatty acids for survival [3]. Lipid loaded macrophages or foamy macrophages accumulate during lipid pneumonia associated with post primary tuberculosis [4]. Sputum analysis of tuberculosis patients from different geographical locations revealed that a subpopulation of the acid fast bacilli in the sputum carried intracytoplasmic lipid bodies [5]. It was further demonstrated that *Mtb* induces the differentiation of macrophages to foamy macrophages and inside the foamy macrophages, the bacilli-containing phagosomes fuse with the cellular lipid bodies [6]. The bacilli then accumulate intracytoplasmic lipid inclusions and stop replicating. Foamy macrophages in mouse granulomas also contain lipid body positive acid fast bacilli [7]. In an *in vitro* multiple stress model, *Mtb* became nonreplicating, nonacid-fast, tolerant to isoniazid and rifampicin, and accumulated triacylglycerol (TAG) and wax ester [8]. A later study showed that while macrophages grown under hypoxic conditions accumulated lipid bodies, the bacilli inside imported fatty acids derived from host TAG for esterification into bacterial TAG [9]. In these hypoxia-induced foamy macrophages the bacilli stopped replicating, frequently lost acid-fast property, and became phenotypically tolerant to isoniazid and rifampicin. The bacilli persisting inside adipocytes are also shown to have these phenotypes [10,11]. Thus, in this context, an adipocyte model offers an alternative to foamy macrophages. Differentiation of normal macrophages to the foamy phenotype either needs very-low-density lipoprotein/low density lipoprotein treatment [12] or provision for hypoxic cell culture [9] which are expensive or sophisticated. However, the adipocyte differentiation of the 3T3L1 adipocyte-like cell line can be achieved by well-established protocols which do not need any special equipment.

While the TAG in the lipid bodies of mycobacteria primarily serves as the source of carbon and energy for the metabolically inert bacilli [9], these and the wax esters may have other yet unknown functions. For example, TAG levels were found to rise in microvesicles secreted by the bacilli under low iron conditions [13]. The bacilli may also synthesize TAG to get rid of the toxic fatty acids in the macrophage compartment. Triacylglycerol synthases (TGS) or diacylglycerol acyltransferases (DGAT) are the enzymes that catalyze the last step in TAG biosynthesis, that is, esterification of the acyl group from acyl-CoA to a diacylglycerol (DAG). There are 15 TGS encoding genes in the *Mtb* H37Rv genome and several of them are induced when the bacilli enter an *in vitro* nonreplicative phase induced by hypoxia and nitrosative stress [14]. Hypoxic cultures of *Mtb* that had accumulated TAG, hydrolyzed the stored TAG when subjected to nutrient starvation [15]. The study identified one of the 24 putative lipases as the major lipase (lipY) involved in TAG degradation during *in vitro* nutrient starvation. Many of the remaining lipases, if not all, may have unique or supporting roles to play in a less defined environment *in vivo*.

Other than these two classes of enzymes, there could be more enzymes which perform these functions in addition to a known function. Ag85A, for example, was known previously as a mycolyltransferase, was recently demonstrated to possess DGAT activity [16]. The *Mtb* genome also consists of seven genes coding for cutinase-like proteins (CULPs). Cutinases, mainly found in phytopathogenic fungi or bacteria, degrade cutin, a polyester that protects plant leaves. However, they are capable of degrading a wide range of other substrates, including carboxylic esters, TAGs, and phospholipids [17,18]. Interestingly, the CULPs of *Mtb* do not degrade cutin, instead function as lipases, esterases, or phospholipases, and hence are thought to serve diverse physiological functions.

The purpose of this study was to determine which of the genes associated to lipid storage and degradation are important to *Mtb* surviving in the host cell amidst the host lipids, using 3T3L1 adipocyte as a model of *ex vivo* dormancy.

Materials and methods

Bacteria and growth conditions

M. tuberculosis H37Rv was grown in Middlebrook 7H9 medium supplemented with 10% oleic acid–albumin–dextrose–catalase (BD, Sparks, MD, USA) and 0.05% tween 80. Cultures were incubated at 37 °C until midexponential phase.

Cell culture and Mtb infection

Mouse 3T3L1 preadipocytes (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, St. Louis, MO, USA), with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and antibiotic antimycotic solution (Sigma-Aldrich, St. Louis, MO, USA) in 5% CO_2 at 37 °C. Cells were grown and differentiated to mature adipocytes in 175 cm^2 tissue culture flasks. Differentiation was performed as recommended by the American Type Culture Collection.

Mtb H37Rv were grown in Middlebrook 7H9 medium (supplemented with 10% oleic acid–albumin–dextrose–catalase, 0.2% glycerol and 0.05% tween 80) to the midlogarithmic phase. Bacteria were declumped using mild sonication before infection. Differentiated adipocytes were infected at a multiplicity of infection of 1:50 for 18 h at 37 °C under 5% CO_2 in Dulbecco's Modified Eagle's Medium containing 10% serum. Extracellular bacilli were removed with amikacin treatment (200 μg/mL) for 2 h and cells were incubated further in fresh culture medium.

RNA extraction and quantitative polymerase chain reaction analysis

At desired time points, *Mtb* infected adipocytes were lysed in QIAzol reagent (Qiagen, Valencia, CA, USA). The lysate was centrifuged at 5000*g* to pellet *Mtb* cells. The pellet was resuspended in QIAzol reagent, transferred in 2 mL screw capped tubes containing MagNA Lyser green beads (Roche Diagnostics, Indianapolis, IN, USA). *Mtb* were disrupted five times for 30 s each in MagNA Lyser instrument (Roche Diagnostics, Indianapolis, IN, USA) with cooling on ice for 1 min after each cycle of cell rupture. The RNA in the lysate was purified using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). To remove DNA contamination, RNA samples were treated with DNaseI

(Thermo Fisher Scientific, Waltham, MA, USA) for 1 h. RNA quantity and quality was quantified using NanoDrop Spectrophotometer (NanoDrop1000, Thermo Scientific Waltham, MA, USA). Similarly RNA was isolated from midlog *Mtb* cells by using Trizol (Thermo Fisher Scientific, Waltham, MA, USA). One microgram of total RNA sample was reverse transcribed in 20 μL reaction volume using RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). Control reactions, lacking reverse transcriptase, were performed for every sample.

The primer sequences are listed in Table S1. Real-time polymerase chain reaction (PCR) primers for the mycobacterial genes were designed using the primer express software (version 2.0.0, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Standard curves were generated by using serial dilutions of H37Rv genomic DNA as template. All the primers were optimized to achieve >90% PCR amplification efficiency and every primer pair was checked by amplifying the unique and the right sized product using the melt curve analysis with Roche 480 II System and software version 1.5 (Roche Diagnostics, Indianapolis, IN, USA). SYBR green-based PCR reactions were accomplished using the Light Cycler 480 II System in a total volume of 10 μL. PCR amplification conditions comprised of an initial cycle of denaturation at 95 °C (4 min), followed by 40 cycles of denaturation (95 °C for 15 s), annealing (optimal temperature for 15 s), and extension (72 °C for 15 s). Reactions were run in triplicates and the specificity of the amplicons was verified using gel electrophoresis and melting curve analysis. For each RNA sample, the target cDNA was normalized internally to the *sigA* (a constitutively expressed *Mtb* gene) cDNA levels in the same sample and relative expression values were calculated using the $2^{-\Delta\Delta CT}$ method [19].

Data analysis

The raw crossing point-PCR-cycle (Cp) values were exported into excel spreadsheet and were used to calculate the fold change in $\log_2$ scale, to determine the relative expression of each gene. *sigA* was used as the reference gene to normalize the Cp values of the target genes and *in vitro* samples was used as the calibrator to calculate fold induction. The *p* values for differences in Cp values were calculated using paired two-tailed *t*-test using Microsoft Excel. All values were means ± standard deviations (SD) from three replicates ($n = 3$).

Results

Altogether, transcriptional changes in 50 *Mtb* genes were analyzed using quantitative PCR during bacterial survival in adipocytes compared to *in vitro* growing bacilli. The entire data is provided in Table S2.

Adaptation to dormancy and metabolic shift

In order to confirm that *Mtb* enters dormancy and undergoes metabolic shift inside adipocytes, the expression of three key genes (*dosR*, *icl*, and *citA*) was analyzed on Day 1 and Day 6 postinfection (Fig. 1). Compared with *in vitro* growing *Mtb*, *dosR* expression was upregulated (6.82 $\log_2$-fold) in adipocyte-dwelling *Mtb* on Day 1 of infection and was further

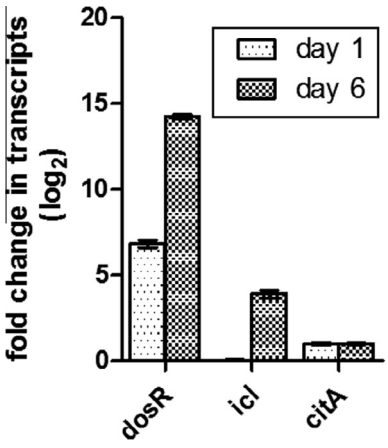

Fig. 1 – Expression of dormancy and metabolic shift genetic markers by adipocyte-dwelling *M. tuberculosis*. Graph shows the expression of three key genes associated to dormancy (*dosR*) and metabolism (*icl* and *citA*) by intra-adipocyte bacilli. The $\log_2$-Fold change for each time-point is relative to *in vitro* growing (exponential phase) bacilli. The values plotted are the mean ± standard deviation of triplicate experiments in three biological replicates.

upregulated on Day 6 (14.23 $\log_2$-fold). The *icl* expression on Day 1 was nearly the same as in the *in vitro* growing bacilli, but was upregulated on Day 6 (3.7 $\log_2$-fold). Compared with *in vitro* growing *Mtb*, *citA* transcripts nearly doubled in adipocyte-dwelling *Mtb* on Day 1 and the levels remained unchanged on Day 6.

Expression of TGS encoding genes

Sixteen *tgs* genes involved or thought to be involved in synthesis of TAGs were analyzed. Six genes were downregulated in adipocytes by >2 $\log_2$-fold at one or both time points (Table 1). The remaining 10 *tgs* genes were upregulated in adipocytes at one or both time points (Fig. 2), out of which eight were upregulated by >2 $\log_2$ ratio. Three genes (*Rv1760*, *Rv3233c*, and *Rv3740c*) were upregulated only at the late time point, while one (*tgs3*) was upregulated initially, but was slightly downregulated by Day 6. The remaining four genes (*tgs1*, *tgs2*, *Rv3371*, and *Rv3804c*) were upregulated at both time points by >2 $\log_2$-fold, with *tgs1* showing the highest upregulation.

Expression of genes encoding lipases, esterases, and CULPs

Among the 24 probable lipase/esterase-encoding genes, seven genes were downregulated by >2 $\log_2$-fold in intra-adipocyte *Mtb* relative to *in vitro* growing *Mtb* (Table 1). Seventeen genes were upregulated either at one or at both time points (Fig. 3), of which 11 were upregulated by >2 $\log_2$-fold. Eight of these (*lipE*, *lipF*, *lipI*, *lipJ*, *lipK*, *lipN*, *lipQ*, and *lipY*) were upregulated by >2 $\log_2$-fold only at the late time point. The remaining three (*lipH*, *lipV*, and *lipX*) were upregulated at both time points. *lipF*, *lipH*, *lipN*, *lipX*, and *lipY* were the highly expressed lipase genes, showing >8 $\log_2$-fold change.

Three out of the seven genes encoding for CULPs were downregulated in intra-adipocyte bacilli relative to *in vitro*

Table 1 – Genes encoding TGS/lipase/CULPs that were down-regulated in intra-adipocyte bacilli compared to *in vitro* logarithmic phase bacilli.

Locus	Gene	Predicted function	Fold change in transcripts (*in vitro* vs. intra-adipocyte)			
			Day 1		Day 6	
			Log_2 ratio	*p*-value	Log_2 ratio	*p*-value
Rv3088	*tgs4*	TGS	−6.02	<0.001	−8.08	<0.001
Rv2285	*Rv2285*	TGS	−1.76	<0.001	−3.18	<0.001
Rv0221	*Rv0221*	TGS	−1.87	0.004	−6.2	<0.001
Rv3087	*Rv3087*	TGS	−1.94	<0.001	−6.26	<0.001
Rv1425	*Rv1425*	TGS	−0.72	<0.001	−5.54	<0.001
Rv0895	*Rv0895*	TGS	−3.12	<0.001	−7.68	<0.001
Rv0220	*lipC*	Esterase	−3.05	<0.001	−4.41	<0.001
Rv1923	*lipD*	Lipase	−3.77	<0.001	−5.27	<0.001
Rv0646c	*lipG*	Lipase/esterase	−3.5	<0.001	−10.33	<0.001
Rv2284	*lipM*	Esterase	−5.96	<0.001	−6.85	<0.001
Rv2463	*lipP*	Lipase/esterase	−3.65	<0.001	−5.15	<0.001
Rv0217c	*lipW*	Esterase	−3.08	<0.001	−6.31	<0.001
Rv1834	*lipZ*	Hydrolase	−3.39	<0.001	−7.61	<0.001
Rv1984c	*culp1 (cfp21)*	Cutinase	−2.51	<0.001	−5.08	<0.001
Rv2301	*culp2 (cfp25)*	Cutinase	−3.54	<0.001	−8.31	<0.001
Rv3452	*culp4*	Cutinase	−1.44	<0.001	−5.08	<0.001

Note: TGS = triacylglycerol synthase.

growing bacilli (Table 1). The remaining four genes (*culp*3, 5, 6, and 7) were upregulated at one or both time points (Fig. 4). *Culp*5 and *culp*7 showed the highest upregulation, especially at the later time point.

Discussion

Lipid bodies, irrespective of the cell type or organism, have a basic architecture comprised of core-containing neutral lipids, mainly TAG and sterol esters, surrounded by a phospholipid hemimembrane with associated proteins. Lipid bodies in immune cells like the macrophages contain arachidonic acid associated with pools of phospholipid and/or neutral lipids, which acts as a key signaling molecule in cell activation [20]. Here we have focused on the transcriptional adaptation of intracellular *Mtb* to store and utilize host lipids and used adipocytes to provide an intracellular niche rich in stored host lipids. We have analyzed 50 genes using quantitative reverse transcriptase PCR. Previously published data is available for only 10 of these genes in *Mtb* inside foamy macrophages [9].

Earlier studies, including ours, have shown that *Mtb* inside mature adipocytes enter nonreplicating persistence and show phenotypic properties characteristic of dormancy [10,11]. In this study a few genotypic markers were included to confirm *Mtb* dormancy in adipocytes/host lipids, by looking at the expression of three key genes (*dosR*, *icl*, and *citA*). DosR/devR, is a two component transcriptional regulatory protein that controls the expression of 48 hypoxia/nitric oxide stress-induced genes [21]. Even though the adipocyte culture is not hypoxic, this gene was analyzed because its expression is highly upregulated in "persisters" in sputum [5]. In addition, the expression of *tgs*1, a prominent TGS responsible for lipid body formation, is DosR-regulated, which emphasizes the involvement of DosR in bacilli on a lipid diet. There was significant upregulated expression of *dosR* from the 1st day of the intra-adipocyte life. This upregulated expression in the absence of hypoxia or nitric oxide stress could have been triggered by the lipid-rich environment, as was reported by a recent study on *Mtb* surviving on long chain fatty acids [22]. DosR, a heme-based sensor protein, has also been reported to respond to reductive stress [23]. It is speculated that metabolic activities of *Mtb* in a lipid-rich environment may cause an abnormal increase in reducing equivalents like hydroxylamine reductase, hydrogenated flavin adenine dinucleotide, or nicotinamide adenine dinucleotide phosphate leading to reductive stress [22].

Microbes growing on acetate or fatty acids as the sole carbon source make use of the glyoxylate bypass for the biosynthesis of cellular material. Isocitrate lyase, one of the key enzymes in the glyoxylate bypass plays a pivotal role in the persistence of *Mtb* in mice by sustaining intracellular infection in inflammatory (activated) macrophages [24]. Expression of *icl* was found to be upregulated in adipocytes on Day 6 and is indicative of bacterial utilization of the glyoxylate shunt.

Citrate synthase encoded by the *citA* gene is the TCA cycle enzyme that incorporates acetyl CoA into the tricarboxylic acid (TCA) cycle. *citA* expression was in fact nearly doubled inside adipocytes compared with that in MB 7H9 broth. The expression was maintained at the same levels, even on Day 6, inside adipocytes. It has been suggested that TAG synthesis by the bacteria competes with TCA cycle as both the pathways utilize acetyl CoA [25]. *Mtb* converts host-derived fatty acids to TAGs which are stored in lipid bodies for deriving energy during dormancy and reactivation [14]. Even though *Mtb* inside adipocytes accumulates lipid bodies [10,11], we did not observe a downregulation of *citA* (and TCA cycle). This could be due to the fact that acetyl CoA is more abundant in a lipid-rich environment than in a limited nutrient broth, thus facilitating both the metabolic pathways.

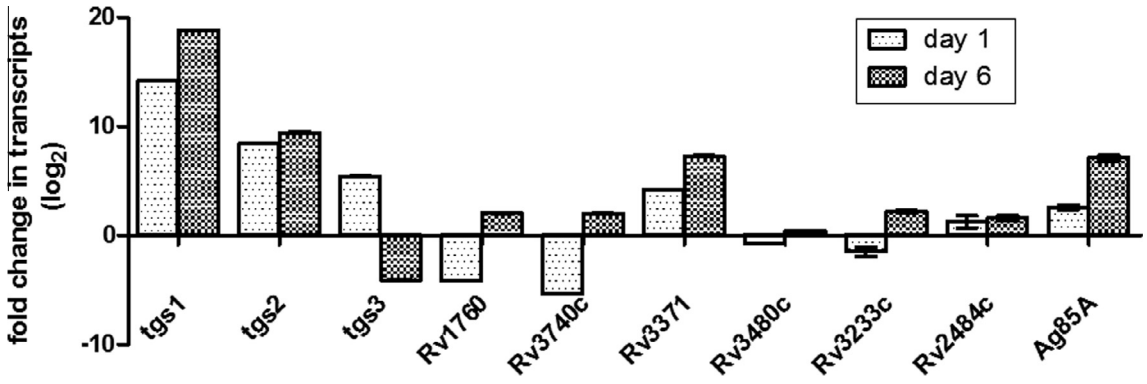

Fig. 2 – Expression profile of triacylglycerol synthase genes that were upregulated in adipocyte-dwelling *M. tuberculosis*. The expression of triacylglycerol synthase genes that were upregulated at least at one time-point during survival in adipocytes are only shown. The log_2-Fold change for each time-point is relative to *in vitro* growing (exponential phase) bacilli. The values plotted are the mean ± standard deviation of triplicate experiments in three biological replicates. The number prefixes are gene locus tag (Rv) numbers for respective *Mtb* genes.

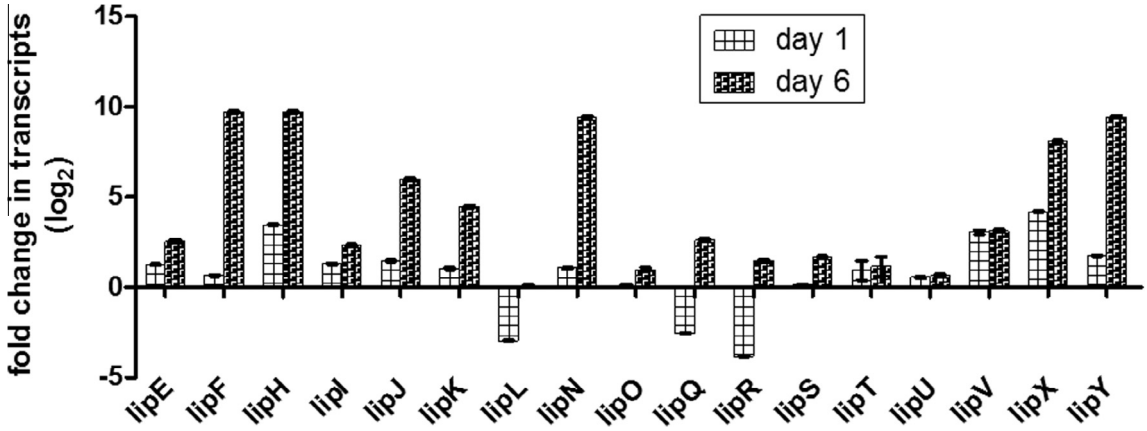

Fig. 3 – Expression profile of genes encoding lipid degrading enzymes that were upregulated in adipocyte-dwelling *M. tuberculosis*. The expression of lipase genes that were upregulated at least at one time-point during survival in adipocytes are only shown. The log_2-Fold change for each time-point is relative to *in vitro* growing (exponential phase) bacilli. The values plotted are the mean ± standard deviation of triplicate experiments in three biological replicates.

When the *in vitro* replicating bacilli entered the lipid-rich cytoplasm of adipocytes, six of the putative TGSs were downregulated, indicating that they are probably not involved in converting host fatty acids to TAG. These enzymes might take part in other cellular processes, most likely cell wall synthesis. It is not uncommon for enzymes to have dual catalytic roles—for instance the mycolyltransferase Ag85A possesses both DGAT and mycolyltransferase activities [16], while BCG1721 (counter-part of Rv1683) has both long-chain acyl-CoA synthase and lipase [26].

Observations suggest that tgs1, tgs2, Rv3371, and mycolyltransferase Ag85A are likely the prominent TGSs involved in TAG synthesis in *Mtb* surviving essentially on total lipids. A few more TGSs may contribute to TAG synthesis in a time dependent manner. Thus, probably tgs3 is likely needed during transition from the replicating to nonreplicating phase, while products of *Rv*1760, *Rv*3233c, and *Rv*3740c are more needed after dormancy is established. Tgs1 and tgs2 have been found associated to lipid droplets in hypoxic, nonreplicating BCG [26]. Daniel et al. [9] used hypoxia to generate lipid-loaded macrophages and analyzed the transcription of some of the TGSs, lipase, and dormancy markers genes. They found elevated expression of *tgs*1, *tgs*4, *Rv*1760, and *Rv*3371 at 72 h relative to 18 h postinfection. The observations match with ours except for tgs4 (Rv3088), which in our study was found downregulated relative to *in vitro* growing bacilli. Both *tgs*1 (*Rv*3130c) and *Rv*3371 were upregulated in *Mtb* growing *in vitro* in presence of long chain fatty acids as the sole carbon source [22]. In a multiple stress model with hypoxia, high CO_2, acidic pH, and low nutrients, *Mtb* showed phenotypic properties of dormancy along with TAG accumulation [8]. In this model, *tgs*1 was the most upregulated of all *tgs* genes and a *tgs*1 mutant failed to make lipid bodies in this model. *Rv*3371 and *Rv*1760 were the other prominent genes induced in this multiple stress model.

Our results suggest that lipF, lipH, lipN, lipX, and lipY are the prominent probable lipases used by intracellular *Mtb* surviving amidst the host fat. Three lipases (lipH, lipV, and lipX)

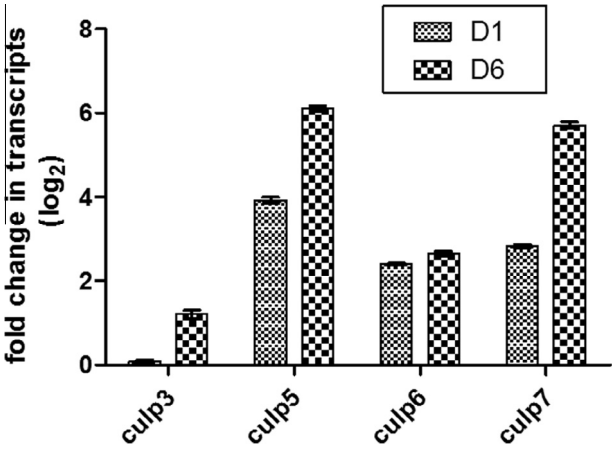

Fig. 4 – Expression profile of genes encoding cutinase-like proteins that were upregulated in adipocyte-dwelling *M. tuberculosis*. The expression of cutinase-like protein genes that were upregulated at least at one time-point during survival in adipocytes are only shown. The log_2-Fold change for each time point is relative to *in vitro* growing (exponential phase) bacilli. The values plotted are the mean ± standard deviation of triplicate experiments in three biological replicates.

are expressed during entry into dormancy and after establishing dormancy, suggesting that they may be used by the bacteria to degrade host TAG to release fatty acids. However, lipE, lipF, lipI, lipJ, lipK, lipN, lipQ, and lipY are probably more crucial after establishing dormancy, for the release of fatty acids from bacterial TAG to derive energy. lipF is a carboxylesterase [27] with additional phospholipase C activity [28] which is essential for *Mtb* survival in mouse [29]. It is induced in early phagosomes of resting macrophages [30]. The *lipF* promoter is acid-inducible [31], which does not respond to stresses like temperature, oxidative stress and hypoxic stress [32]. A clear upregulation (9.7 log_2-fold) inside adipocytes on Day 6 could be due to a possible drop in intra-adipocyte pH as a consequence of increased lipolysis releasing free fatty acids [33]. The proposed function of lipF is to help the *Mtb* survive acid stress by either modifying the cell wall or release metabolic energy from fat to pump protons out of the mycobacterial cytosol in an acidic surrounding [32]. lipY was the most highly expressed lipase by *in vitro* dormant *Mtb* under nutrient starvation [15], suggesting that lipY is probably the predominant lipase involved in hydrolyzing stored bacterial TAG. Induction of more lipases in the adipocyte model suggests that within the host cell multiple lipases may be used by dormant or reactivating *Mtb* to degrade different TAG species. Lipase genes that were downregulated intracellularly compared with *in vitro* conditions might be involved in cell wall synthesis.

None of the cutinase-like enzymes of *Mtb* cleaves cutin, instead function as esterases or lipases of differential preferences for substrate chain length, involved in lipid metabolism or cell wall synthesis [17]. *Mtb* inside adipocytes expressed four genes encoding cutinase-like proteins (*culps* 3, 5, 6, and 7). Culp6 has been reported as a cell wall associated lipase, probably essential for growth and a strong T-cell antigen [34]. Culp5 and culp7 are probably cytoplasmic enzymes, which are yet to be characterized. Interestingly, bioinformatic analysis suggests that some of the *culp* genes are products of gene duplication, for instance, the pairs Culp3/Culp4 and Culp7/Culp5 [17].

In conclusion, *Mtb* expresses different subsets of genes encoding TGS, lipase, and CULPs during active growth *in vitro* and intracellular in the presence of host neutral lipid stores. This suggests that some of these enzymes are probably involved in cell wall synthesis, while some in the utilization of host lipids.

Conflicts of interest

None to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijmyco.2015.10.003.

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