Biochemical Studies on Redox Regulation in Different Dormancy Models of Mycobacteria

Thesis Submitted to
Savitribai Phule Pune University

For The Degree Of

DOCTOR OF PHILOSOPHY IN BIOTECHNOLOGY

By

Ketaki Dilip Shurpali

Research Supervisor

Dr. Dhiman Sarkar

Combichem Bioresource Center

Organic Chemistry Division

CSIR-National Chemical Laboratory

Pune-411008

India

October 2014

Certificate

This is to certify that the work incorporated in the thesis entitled "**Biochemical Studies on Redox Regulation in Different Dormancy Models of Mycobacteria**" submitted by **Ketaki Dilip Shurpali** was carried out under my supervision at Combichem Bioresource Center, Organic Chemistry Division, National Chemical Laboratory, Pune-411008, Maharashtra, India. Materials obtained from other sources have been duly acknowledged in the thesis.

Dr. Dhiman Sarkar

(Research Guide)

Declaration by Research Scholar

I hereby declare that the thesis entitled "**Biochemical Studies on Redox Regulation in Different Dormancy Models of Mycobacteria**", submitted for the Degree of *Doctor of Philosophy* to the Savitribai Phule Pune University, has been carried out by me at Combichem Bioresource Center, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune-411008, Maharashtra, India, under the supervision of Dr. Dhiman Sarkar (Research supervisor). The work is original and has not been submitted in part or full by me for any other degree or diploma to any other University.

Ketaki Dilip Shurpali

(Research Scholar)

Dedications

This thesis is dedicated to all those people who were always besides me in my good and bad times. First dedication goes to my father Mr. Dilip Shurpali and mother Mrs. Devyani Shurpali who instilled in me the desire to learn new things and confidence to achieve my dreams. Second, to my dear husband Kunal for never doubting my ability to achieve a goal and has always helped me by all possible means he could and my little sister Saniya who has made my journey enjoyable and colourful. Third, to my "one more" lovely parents, Mr. Prakash Pokale and Mrs. Shobha Pokale for being very supportive of every endeavour I have ventured to take especially at the end of my thesis. All these people have made my thesis possible.

Ketaki Dilip Shurpali

All truths are easy to understand once they are discovered; the point is to discover them.

Galileo Galilei

Acknowledgements

Though only my name appears on the cover of this dissertation, a great many people have contributed to its completion. I have been blessedby Almighty with some extraordinary people who have spun a web of support around me. I owe my gratitude to all those people who have made this dissertation possible and because of whom my PhD experience has been one that I will cherish forever.

I am deeply indebted to my research supervisor, Dr. Dhiman Sarkar who is not only a great mentor but also a very good human being. I thank him for his limitless patience, help, encouragement and guidance he has given me till now and I wish it continues forever. Each meeting with him has added invaluable aspects to the implementation and broadened my perspective. He has guided me with his invaluable suggestions, lightened up the way when things seemed obscure and encouraged me a lot in the academic life. From him I have learned to think critically, to select problems, to solve them and to present their solutions. I thank him for giving me freedom and resources to pursue different lines of research. I will miss eating his brain for answers to my questions. It was a great pleasure for me to have a chance of working with him. I wish him continuing success in his future endeavours.

I thank CSIR, New Delhi for providing me Research Fellowship to carry out my work for PhD.I consider it a great honour to have been part of the CSIR-National Chemical laboratory. This has been the best time I have spent in my life where I have learnt a lot more than just research. I am highly obliged to Director NCL, Dr. Sourav pal and our HOD, Dr. Pradeep Tripathi for their excellent leadership and an inspirational working environment they have provided in the institute.

I am also thankful for the guidance and encouragement I have received from my PhD research committee members Prof. J. K. Pal, Dr. Vidya Gupta and Dr. C.G. Suresh.

I also want to thank Dr. Mahesh Kulkarni and Yashwant for their help during proteomics work and Dr. Sen for teaching me how to make samples for Scanning Electron Microscopy. I also want to thank Dr. Thulasiram, Dr. M.V. Deshpande, Dr. R.A. Joshi and Dr. (Mrs.) R.R. Joshi for their help. I want to extend my thanks to all the staff members from Stores & Purchase section, Accounts section, Bill section,

OCD office and SAC office for helping me all the time for the official work. I want to thank Dr. Mitra from NCCS for allowing me to use his Real Time PCR machine and Priyanka for helping me out in my experiment.

Special thanks goes to my colleagues Upasana, Sampa, Abhishek, Shamim, Manoj, Laxman, Amar, Vijay, Nandadeep and Hardik for the help I have received countless number of times from them. I was fortunate to work with Nikita during last two years of my PhD, from whom I have learnt a lot. I specially want to thank Meghana, Manisha and Suwarna for being always positive and supportive especially during my thesis writing and helped me correct my thesis drafts. I consider it a great privilege to have been associated with such amazing "budding" scientists.

Thanks to all my other friends who have always given me happiness and encouragement. I specially want to thank Herschel for always giving me right suggestions at right time. The list would be incomplete without one more person from NCL, Pooja, who made my PhD journey so joyous and I will always cherish the time we have spent together.

Words fall short if I try to thank my dear husband, Kunal for the unreserved support I have received from him. His perfection and sincerity have been my inspiration. I specially want to thank my sweet little sister, Saniya for always being there for me and loving me unconditionally. I am blessed with the unconditional love I have received from my nephew, Aarya. I give all the credits to Aarya and Dad for making me forget all my tensions and worries after coming home. I take this opportunity to thank Nikhil and Trupti for being so supportive and considerate during the crucial final years of my thesis. Another important person who has made this journey joyous and easy is Uncle; I really want to thank him. The infallible love and encouragement I have received from the entire Pokale, Shurpali and Aphale families have always been my strengths. Finally, this would not have been possible without the support of my two lovely parents, Dilip & Devyani Shurpali and Prakash & Shobha Pokale and my dear husband Kunal. I feel blessed to have such a wonderful family.

Ketaki Dilip Shurpali

Table of Contents

Abbreviations

Sr. No.	2-MP	2-Mercaptopyridine
1	AA	Ascorbic Acid
2	ABC	Ammonium bicarbonate
3	Ahp	Alkyl Hydroperoxide Reductases
4	AhpC	Alkyl hydroperoxidase C
5	AIDS	Acquired Immunodeficiency Syndrome
6	BCG	Bacilli Calmette-Guerin
7	c-DNA	Complementary DNA
8	CFP-10	Culture Filtrate Protein-10
9	CFU	Colony Forming Unit
10	c-RNA	Complementary RNA
11	DCFH-DA	2,7-Dichlorodihydro fluorescein diacetate
12	DCF	2',7'-dichlorofluorescein
13	DHE	Dihydroethidium
14	DMSO	Dimethylsulfoxide
15	DOT	Directly Observed Treatment
16	DPI	Diphenyleneiodonium Chloride
17	DTPA	Diethylene triamine pentaacetic acid
18	DTT	Dithiothreitol
19	ELISA	Enzyme Linked-ImmunoSorbent Assay
20	EMB	Ethambutol
21	ESAT-6	Early Secretary Antigen Target-6
22	ETC	Electron Transport Chain
23	FPR	False Positive Rate
24	GFP	Green Fluorescent Protein
25	GPLs	Glycopeptidolipids
26	H_2O_2	Hydrogen Peroxide
27	HPLC	High Performance Liquid Chromatography
28	HSR	Head Space Ratio
29	ICL	Isocitrate lyase
30	IFN	Interferon
31	INH	Isoniazid
32	KatG	Catalase peroxidase
33	LAM	Lipoarabinomanan
34	LC/MS^E	Liquid chromatography-tandem mass spectrometry
35	Lpd	Dihydrolipoamide dehydrogenase
36	MDR	Extremely Drug Resistant
37	MHC-II	Major Histocompatibility Complex Class II

38	Msr	Methionine Sulfoxide Reductase
39	Mtb	*Mycobacterium tuberculosis*
40	MW	Molecular Weight
41	NADH	Nicotinamine adenine dinucleotide (reduced)
42	NADPH	Nicotinamine adenine dinucleotide phosphate (reduced)
43	NBT	Nitro Blue Tetrazolium
44	NHP	Non Human Primate
45	NO	Nitric Oxide
46	NRP-1	Non-replicating Persistence Stage 1
47	NRP-2	Non-replicating Persistence Stage 2
48	OD	Optical Density
49	PAS	p-Amino Salicylic Acid
50	PBS	Phosphate Buffered Saline
51	PCR	Polymerase Chain Reaction
52	PLGS	Protein Lynx Global Server
53	PMF	Proton Motive Force
54	PPD	Purified Protein Derivative
55	PZA	Pyrazinamide
56	Q-Tof	Quadrupole Mass Analyzer- Time of flight
57	RD	Region Of Differentiation
58	RIF	Rifampicin
59	RNS	Reactive Nitrogen Species
60	ROS	Reactive Oxygen Species
61	RPM	Revolutions Per Minute
62	SD	Standard Deviation of Mean
63	SodA	Superoxide dismutase A
64	SodC	Superoxide dismutase C
65	sSNP	Synonymous Single Nucleotide Polymorphisms
66	SucB	Dihydrolipoamide Succinyltransferase B
67	TB	Tuberculosis
68	TCA	Tricarboxylic acid Cycle
69	TFA	Trifluoroacetic acid
70	TLR	Toll-like receptors
71	trHbs	Truncated Haemoglobins
72	Trx	Thioredoxin Reductase
73	TST	Tuberculin Skin Test
74	UPLC	Ultra Pressure Liquid Chromatography
75	UV	Ultra violet
76	WHO	World Health Organisation
77	XDR	Multiple Drug Resistant

List of Figures

List of Tables

Abstract

The ability of *Mycobacterium tuberculosis*(Mtb)to persist within humans for long periods with no evident disease symptoms makes it a successful pathogen.It has been estimated that one-third of the Earth's population is 'latently' infected with *Mycobacterium tuberculosis*. One of the major problems of Tuberculosis (TB) management is the incidence of high relapse rate which could be ascribed to a number of reasons. The most important of them is the ability of the microbe to persist in a latent state.The prominent characteristic of latency is resistance to existing drugs. There is still no specific drug available, which could effectively kill this latent bacillus.To develop new drugs against the persistent state of mycobacteria, we should know the precise nature of these dormant forms. This thesis sheds some light on the understanding of the differences at biochemical, transcriptional and translational level between active and latent stages of mycobacteria. Also, it proposes a new mechanism to achieve dormancy in mycobacteria.

Recent studies have highlighted the significant role of ROS as physiological regulators of intracellular signalling pathways. ROS mediated signalling is controlled by a delicate balance between its formation and scavenging. Although, ROS generation by the host has been studied well in mycobacterial infection, its generation by the mycobacteria itself has never received due attention. Its physiological role in growth of the bacteria and development of dormancy needs further research.

We used *Mycobacterium smegmatis* as a model system as it follows many of the key features seen in Mtb, together with having the advantage of being a non-pathogenic and fast growing organism. We demonstrate that *M. sm*egmatis actively produces superoxide radicals under aerobic condition. This was evaluated by XTT (Tetrazolium salt) and DHE (Dihydroethidium). Reduction of DHE gives two products namely, 2-hydroxyethidium, specific for superoxide and a non specific product, ethidium. Chromatographic separation and quantification of oxyethidium by HPLC is the most advanced and accurate method of determining superoxide production. With the help of different ROS modulators and inhibitors, we identified NADH oxidase as possibly the major source of superoxide both in active as well as dormant stage of *M. smegmatis*. As NADH oxidase is a membrane bound enzyme

bacterial membrane was identified as the site of superoxide formation. We also compared ROS generation in active and dormant *M. smegmatis* cells. Wayne's in vitro hypoxia model and Nutrient starvation model were used to obtain dormant cells. We observed less ROS generation in Nutrient Starvation model than in aerobically growing cells but surprisingly, more ROS generation as well as increased $NADH/NAD^+$ ratio was seen in Wayne's in vitro hypoxia dormant model than in aerobically growing cells. We hypothesize that the apparent burst of ROS observed in Wayne's dormancy models could play important role in resuscitation of these bacilli. Further research on this will make the scenario clearer.

As NADH oxidase was found as the major source of superoxide, we analysed the effect of inhibition of NADH oxidase using its inhibitor, DPI on the growth of *M. smegmatis* cells. Inhibition of superoxide in the *M. smegmatis* culture induced bacteriostasis characteristic of dormancy supported by the induction of the DosRS regulon genes, decrease in cell size, change in acid fast staining property and resistance to antibiotics. We have developed a model based on this mechanism which can be used to make dormant *M. smegmatis* cells. The direct link between superoxide withdrawal and induction of dormancy in the bacilli was further confirmed by resuscitation of the dormant bacilli by addition of exogenous superoxide using pyrogallol, a superoxide generator. Non-culturability of dormant cells is the highlight of this model. This model can be used to develop new assays for screening of drugs against the latent form of mycobacteria.

To characterize the DPI induced dormancy model at transcriptional and translational level, gene expression profile as well as protein profile of DPI induced dormancy model was compared with known and established dormancy models namely, Wayne's in vitro gradual oxygen depletion model and ascorbic acid induced dormancy model. The expression ratios were calculated with respect to aerobically growing *M. smegmatis* bacilli which served as a control. 762 genes were found to be up-regulated and 1362 were found to be down-regulatedunder all three conditions. Thus, high number of common genes which were up/down regulated shows similarity between different dormancy models. The number of genes down regulated was higher than the number of genes up regulated which shows overall shut down of cell processes under dormancy. In $LCMS^E$ studies, 24 proteins were found to be up-regulated and 29 were found to be down-regulatedunder all three conditions.We

identified 367 genes and 303 proteins which were up-regulated exclusively in DPI induced dormancy model.This study mainly helps in understanding the metabolic shift during dormancy. Further, we have identified several genes/proteins that were unique/abundant in DPI induced dormancy model such as many transporters, transcriptional regulators etc. Their role in survival of the dormant stage needs further research. This study also supports the identification of potential drug targets against latent tuberculosis which is the need of the hour.

Altogether this thesis provides an insight into molecular mechanisms of latency and resuscitation of *M. smegmatis*. It sheds light on the importance of the study of superoxide generation by mycobacteria and its physiological role during growth and dormancy.

Publications (from Thesis)

- "Evidence of Superoxide Production and its Involvement in Growth of *Mycobacterium smegmatis"***Ketaki D. Shurpali**, Nikita O. Fernandes, Abhishek Mishra, Meghana C. Athalye (Manuscript submitted).

- "Development of a novel, non-cultivable dormancy model by modulating superoxide production in *Mycobacterium smegmatis"*. **Ketaki D. Shurpali,** Nikita Fernandes and Amar Yeware (Manuscript under preparation).

- "Comparison of ROS in active and dormant forms of *M. smegmatis*" **Ketaki D. Shurpali** and Amar Yeware (Manuscript under preparation).

Publications

- "Advanced cell-based high-throughput screening assays targeting active and latent *Mycobacterium tuberculosis"***Ketaki Dilip Shurpali**, Upasana Singh, Shamim Akhtar, Sampa Sarkar, Abhishek Mishra, Roshan Kulkarni, Swati Joshi and Dhiman Sarkar, Journal of Biotechnology, Volume 185 Supplement, 2014, Pg S18.

- "Phyllocladane diterpenes from Anisomeles heyneana" Roshan R. Kulkarni, **Ketaki Shurpali**, Rupesh L. Gawde, Dhiman Sarkar, Vedavati G. Puranik and Swati P. Joshi, Asian. J. Nat. Prod. Res., 2012, 1–7.

- "Chemical investigation of Plectranthus mollis" Roshan R. Kulkarni**, Ketaki Shurpali**, Rupesh L.Gawde, Dhiman Sarkar, Vedavati G. Puranik and Swati P. Joshi ;Journal of medicinal and aromatic plant sciences- 34 (3-4),2012.

- "Labdane Diterpenes with Anti-mycobacterial Activity from Leucas stelligera" Kulkarni Roshan; **Shurpali Ketaki**; Puranik Vedavati; Sarkar Dhiman; Joshi Swati Journal of Natural Products, 2013.

- "Antimycobacterial compounds from *Anisochilus verticillatus* from the Western Ghats" Roshan R. Kulkarni, **Ketaki Shurpali**, Vijay Khedkar, Dhiman Sarkar, Vedavati G. Puranik and Swati P. Joshi, (Manuscript submitted).

Patents

- Antitubercular corsolic acid and 5-hydroxy-6,7,3',4'-tetramethoxyflavone. Inventors: S. P. Joshi, R. R. Kulkarni, D. Sarkar, S. Sarkar**, K. Shurpali** PCT Int. Appl. (2013), WO 2013021258 A1 20130214.

- Phyllocladane diterpene and phenylethyl glycoside compounds from Anisomeles heynaeana with anti-mycobacterial or antiproliferative activity. Inventors: S. P. Joshi, R. R. Kulkarni, D. Sarkar, S. Sarkar**, K. Shurpali** PCT Int. Appl. (2013), WO 2013021260 A2 20130214.

- C-9 functionalized labdane derivatives. Inventors: S. P. Joshi, R. R. Kulkarni, D. Sarkar, S. Sarkar, **K. Shurpali** PCT Int. Appl. (2013), WO 2013024493 A1 20130221.

- Antituberculosis compositions of Byttneria species. Inventors: D. Sarkar, S. P. Joshi., U. Singh, **K. Shurpali**., R. Kulkarni Publication no (2011) WO/2011/117708.

CHAPTER 1

Overview of Tuberculosis

1 INTRODUCTION

1.1 Overview of Tuberculosis

1.1.1 Classification of *Mycobacterium tuberculosis*

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* which can be classified as follows:

Kingdom: Bacteria

Phylum: Actinobacteria

Class: Actinobacteridae

Order: Actinomycetales

Suborder: Corynebacterineae

Family: Mycobacteriaceae

Genus: *Mycobacterium*

Species: *Mycobacterium tuberculosis*

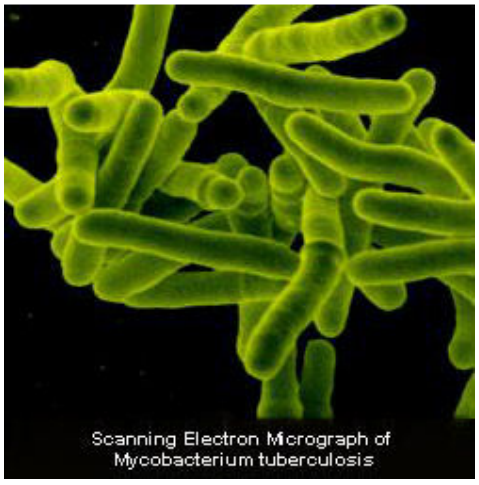


Figure 1.1: Scanning electron Micrograph of M. tuberculosis. Extracted from: http://medicineworld.org/images/blogs/11-2007/mycobacterium-tuberculosis-299290.jpg

1.1.2 Characteristics of *Mycobacterium tuberculosis*

The causative agent of TB, *Mycobacterium tuberculosis* was first discovered in 1882 by Robert Koch. It is a slow-growing organism. The host of this organism is macrophages (Figure: 1.1) [1]. It is acid fast, rod-shaped, non-motile, non-spore forming, and aerobic. The cell wall of Mtb is unique and impermeable to a number of compounds which makes it resistant to numerous drugs [2]. Cell wall is made up of mycolic acid, which gives Mtb the acid fast character and able to retain basic dyes in the presence of acid alcohol. The detailed characteristic features of the mycobacterial cell wall include the lipoarabinomannan (LAM), lipomannan, mycolylarabinogalactan, phosphatidyl-myoinositolmannoside, sulfatide, cord factor, and other acylatedtrehaloses, phenolic glycolipids, lipoligosaccharides, and other attenuated lipids (Figure 1.2). These components are non-covalently attached to the plasma membrane through their GPI anchors and they expand to the outer of the cell wall [3, 4]. LAM is identified as one of the virulence factor of Mtb. It interferes with the phagosomal maturation and cell signalling pathways [3, 5-8]. Mannose-capped LAM (ManLAM) is present in the cell wall of virulent, slow-growing mycobacteria whereas AraLAM or phospho-myo-inositol-capped LAM (PILAM) is found in rapidly growing non-virulent species of mycobacteria such as *M. smegmatis* [9]. The 19-kDa lipoproteins of Mtb which participate in virulence as well as LAM and AraLAM from rapidly growing mycobacteria binds to Toll-like receptors (TLR) on the host cell surface and thus induce an inflammatory response [10-12]. Many of these characteristic features play a major role in the virulence and pathogenesis of the bacillus. The genomic DNA of Mtb possesses high guanosine plus cytosine (G + C) content, a characteristic feature of Mycobacterium sp. Mtb genome has been sequenced and shown to be 4.4 Mb in size and contains 4006 protein-coding genes of which 52% have assigned functions .Out of these, only 376 putative proteins share no homology with known proteins and presumably are unique to Mtb [13]. The availability of its genome sequence facilitates identification of genes encoding various functions including virulence factors as well as protective antigens. It also provides information for identification of targets for drug and vaccine development.

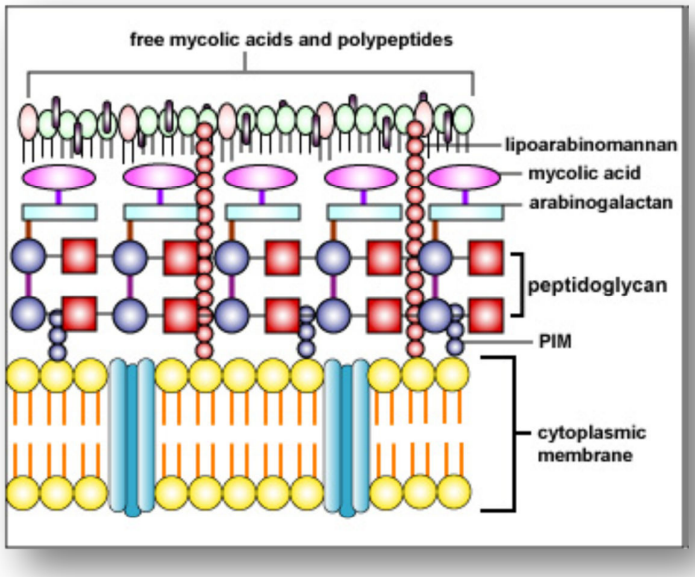


Figure 1.2: Overview of the structure of the cell wall of *M. tuberculosis.* Extracted from Nature Reviews, Microbiology, **5**, 883-891.

The outermost layer of the mycobacterial cell envelope is considered to be a loosely attached capsule composed primarily of glucans, arabinomannans and mannans. Mycobacterial glucan is a branched polymer of D-glucosyl residues with branch points consisting of monoglucosyl to pentaglucosyl substitutions. The arabinomannan consists of a heteropolymer of D-arabino-D-mannan. Structurally the arabinan found in the mycobacterial capsule is similar to the arabinan found in the arabinogalactan portion of the cell wall. The capsular mannan is a branched polymer of D-mannosyl subunits with branch points consisting of single D-mannosyl units. Lipids and proteins are also known to ornament the capsule, albeit in smaller amounts. Capsular proteins fall in two categories: the 'secreted' proteins and 'resident' capsular proteins. Both types of protein are found to be present in culture filtrate indicating that the 'resident' capsular proteins are likely shed from the cell surface into the culture medium. Lipids comprise only 2-3% of the capsular material. Since mycobacteria are cultured in media containing dispersing agents, such as Tween-80, this capsule is frequently lost. Capsular lipids are most commonly glycopeptidolipids (GPLs) [14].

1.1.3 Characteristics of *Mycobacterium smegmatis,* a model organism to study pathogenic mycobacteria

M. smegmatis was first discovered in 1884 by Lustgarten [15-16]. It is predominantly a non-pathogenic environmental saprophyte, but can cause opportunistic subcutaneous infections at sites of cuts and abrasions which are often exposed to soil, generally exploiting circumstances of an immuno-compromised host [17].

M. smegmatis cells are aerobic, non-motile, rod shaped and approximately 3.0-5.0 μm in length by 0.5-1.0 μm in width with a doubling time of 2-3 hours [18]. This bacterium has a tendency to form aggregates in liquid media [19]. Similar to Mtb, these bacteria are also acid fast in nature. *M. smegmatis* genomewas sequenced in 2006 by the J. Craig Venter Institute. It possesses 6,988,209 nucleotides with a 67% GC content which codes for 6716 proteins. Among these over 2000 share close homology with genes of *M. tuberculosis* [20]. *M. smegmatis* strain MC^2155 is has many advantages like, it is hyper-transformable, readily accept foreign plasmid DNA via electroporative transformation, it has relatively fast generation time and broad media requirements. These characteristics have made it the workhorse of modern mycobacterial genetics [21]. Thus, all these properties along with the non pathogenic nature make it a very good model organism to study Mtb [22, 23]. Additionally, it possesses a functional Dos regulon and has been shown to enter dormancy by oxygen depletion as well as nutrient starvation conditions[24]. This makes *M. smegmatis* a relevant screening model for identifying drugs active toward dormant mycobacteria such as persistent *M. tuberculosis*.

1.2 Epidemiology of Tuberculosis

In 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease (including 320 000 deaths among HIV-positive people) [25].

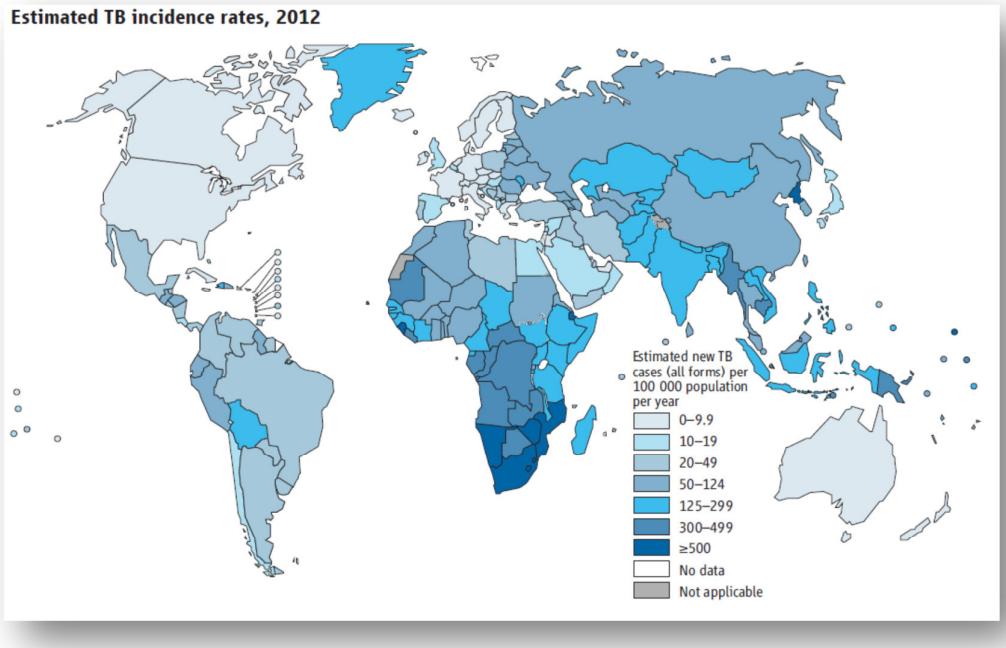


Figure 1.3: .Estimated TB incidence Rates. Extracted from WHO Global Tuberculosis report, 2013.

The prevalence of bacteriologically confirmed pulmonary TB can be directly measured in nationwide population-based surveys using sample sizes of around 50 000 people. Trends in disease burden are assessed by conducting regular repeat surveys after every 10 years. Countries in which surveys have been implemented or are planned in the near future are shown in Figure 1.4 [25].

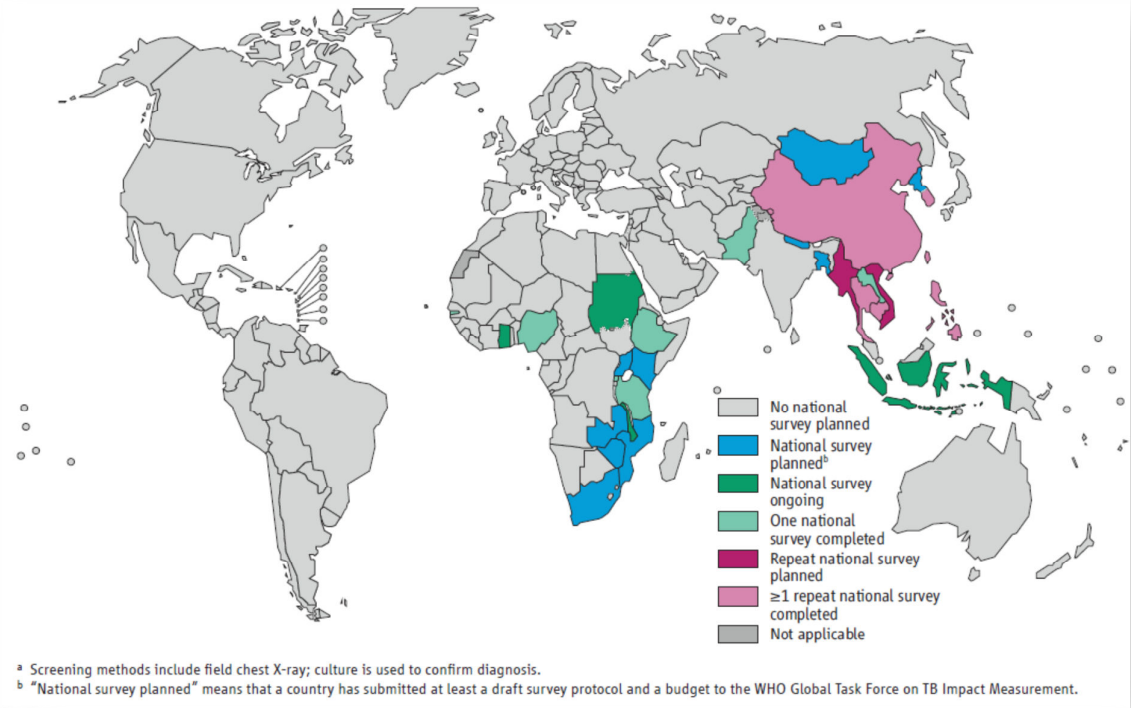


Figure 1.4: Countries in which national population-based surveys of the prevalence of TB disease have been implemented using currently recommended screening and diagnostic methods since 1990 or are planned in the near future: status in July 2013.Extracted from Global Tuberculosis Report, 2013.

1.2 Pathogenesis of *Mycobacterium tuberculosis*

M. tuberculosis enters the alveolar passages of exposed humans in the form of aerosol droplets [26]. After entry, the bacteria are ingested by alveolar macrophages [27, 28]. Additionally, dendritic cells as well as pneumocytes which are better antigen presenters than macrophages can also ingest mycobacteria [29] and thus are presumed to play a key role in activating T cells with specific *M. tuberculosis* antigens [30, 31]. Once, mycobacteria are inside the macrophages, they reside in the endocytic vacuole, phagosome [32]. After entry, if the phagosome-lysosome fusion takes place, the bacteria are bombarded with a variety of ROS, RNS, lysosomal enzymes and toxic peptides. Since most macrophage killing of bacteria occurs in the phagolysosome [33]. But, intracellular pathogens like Mtb have been able to avoid this hostile microenvironment. Much of the study is focussed on understanding the mechanism of prevention of this fusion [34-39] but still it is not clear whether this blocking of fusion is essential for the organism or not. Involvement of Rab proteins, small GTPases, as

well as coronins has been shown in the maturation of phagosomes [40-42]. The mechanism by which virulent mycobacteria prevent phagosomal maturation is not known.

Infected macrophages in the lung attract inactivated monocytes, lymphocytes, and neutrophils by secreting various chemokines [43]. But, all these cells are unable to kill mycobacteria. Thus, granulomatous focal lesions composed of macrophage derived giant cells and lymphocytes are formed. This is the best way to contain the disease and spread of bacilli. After development of cellular immunity, macrophages infected with pathogens are killed and the caseous centre of the granuloma is formed surrounded by a cellular zone of fibroblasts, lymphocytes, and blood-derived monocytes [44]**.** Mtb is unable to multiply within this granulomatous lesion but it can withstand the conditions and remain dormant for decades. In some cases, the infection is arrested permanently but in some it can lead to the next stage, which depends on the immune system of the host. Sometimes, when the immune system of latently infected person drops by various reasons the granuloma can liquefy and thus serve as rich media to help the bacteria grow once again. This uncontrollable growth can lead Mtb to escape from the granuloma and spread within lungs (active pulmonary TB) and evento other tissues via the lymphatic system and the blood (miliary or extra pulmonary TB). When this happens, the person becomes infectious and requires antibiotic therapy to survive**.**

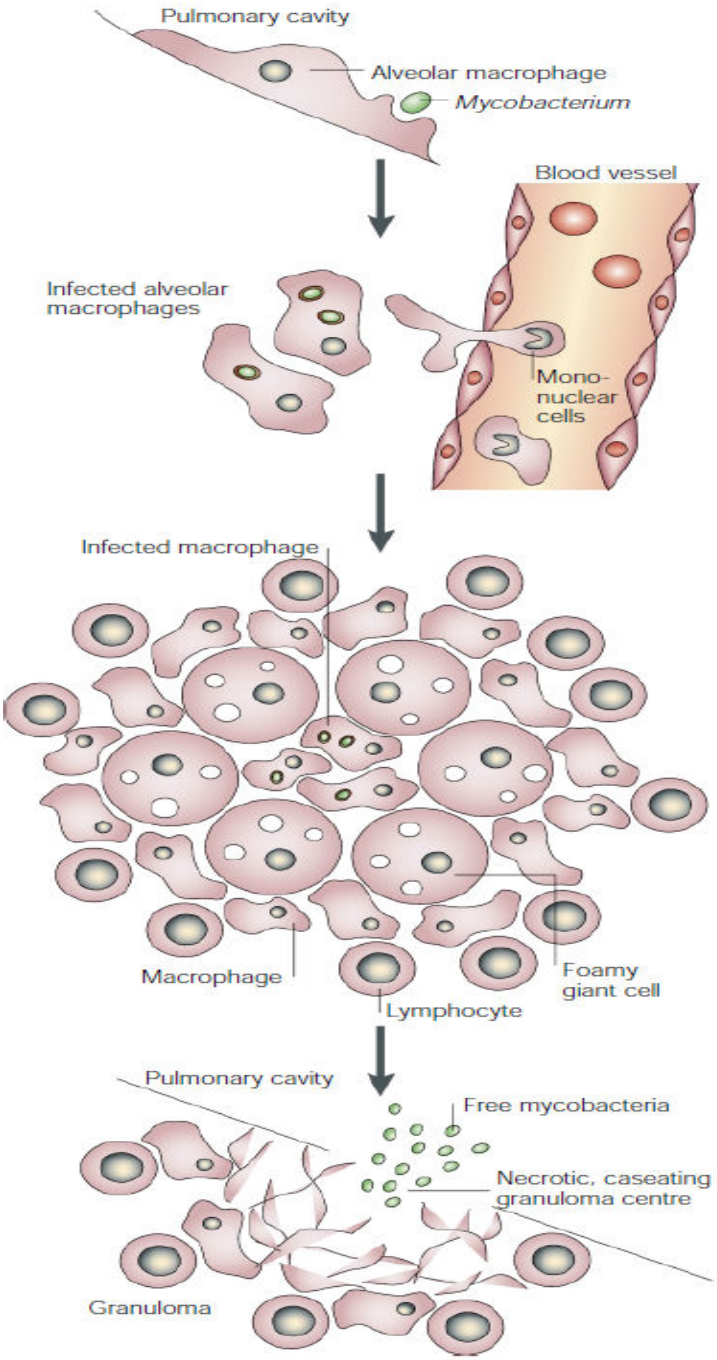


Figure 1.5: Schematic presentation of the progression of *M. tuberculosis* infection. Extracted from *Nature* Reviews, Molecular Cell Biology,**2**, 569-586.

1.4 Oxidative Stress

The interaction between the intracellular pathogen *Mycobacterium tuberculosis* and mononuclear phagocytes typically leads to induction of host adaptive immune responses, which control infection without sterilizing the host. The intracellular environment of host macrophages generates a high output of ROS and RNS. ROS is also produced during bacterial aerobic metabolism [45]. The term RNS includes molecules like NO^-, $^{\bullet}NO_2$, NO_2^-, N_2O_3, N_2O_4, *S*-nitrosothiols, peroxynitrite ($OONO^-$), and dinitrosyl-iron complexes. The term ROS include molecules like superoxide (O_2^-) hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$), and reactive products of these with halides and amines. All these radicals can damage DNA and many other moieties like Fe-S clusters, tyrosyl radicals, hemes, sulfhydryls, thioethers, and alkenes. Peroxynitrite, the additive product of superoxide and nitric oxide can be even more destructive than the two individually [46].

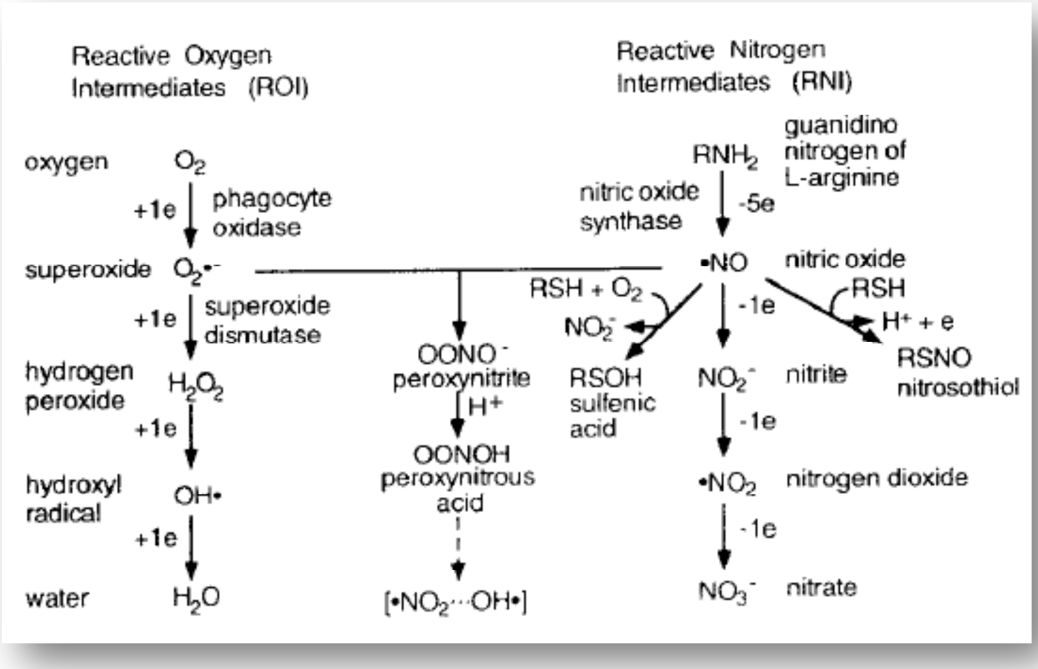


Figure 1.6: ROS and RNS production in mammalian cells. Extracted from PNAS, 97:8841-8848, 2000.

1.5 Strategies to Combat Oxidative Stress

Detoxification of RNS and ROS can be done by various ways in Mtb. First, superoxide can be directly detoxified by the sequential activity of superoxide dismutases (SODs) and catalase (KatG); peroxynitrite can be detoxified by KatG. Second, NO can be directly detoxified by haemoglobins. Third, hydrogen peroxide and peroxynitrite can also be detoxified by thiol-specific redox systems, which also maintain the intracellular thiol-disulfide balance. These include alkylhydroperoxide reductases (AhpC and AhpD), thioredoxin and Thioredoxin reductase (TrxB2) and methionine and methionine sulfoxide reductase (MsrA) [45]. Dihydrolipoamide dehydrogenase (Lpd) and dihydrolipoamide succinyltransferase (SucB) are components of a-ketoacid dehydrogenase complexes that are central to intermediary metabolism. Lpd and SucB support Mtb's antioxidant defence. The peroxiredoxin alkyl hydroperoxide reductase (AhpC) is linked to Lpd and SucB by an adaptor protein, AhpD. AhpD thus represents a class of Thioredoxin-like molecules that enables an antioxidant defence [47]. Mycothiol, a functional analogue of glutathione has been reported as essential for the growth of Mtb by protecting it from oxidative stress [48]. In *M. smegmatis* too, it was observed that mycothiol deficient mutant strains are more sensitive to NO and H_2O_2 mediated toxicity than the wild type cells [49].

On the contrary to the belief that an oxidative environment helps to kill bacterial organisms, tubercular lesions are often present in areas with high tissue pO_2, such as lung apices, indicating that *M. tuberculosis* preferentially grows in oxidative environments [50]. Earlier it is reported that *Allicin*, an antioxidant from *Allium sativum*, reduced *M. tuberculosis* survival in macrophages [51]. An antioxidant mimetic, MnTE-2-PyP, inhibits the intracellular growth of *M. abscessus*, a non-tuberculous intracellular pathogen that resides within macrophages and fibroblast cells of lung and skin [52]. Nitric oxide induced dormancy is well documented, Flynn et al. demonstrated that *M. tuberculosis* reactivation occurs if the production of RNS is inhibited in a murine model of latency [53]. Very recently, an antioxidant, vitamin-C has been shown to have wide ranging regulatory effects on the gene regulation and physiology of *M. tuberculosis* which leads to a dormancy phenotype development and growth arrest [64].

Together these studies set a precedence suggesting that oxidative stress induced by infection may be more detrimental to the host than facilitative in killing bacteria. An oxidizing environment may be well tolerated by intracellular pathogens and potentially necessary for their survival within the host cells [52]. But, how Mycobacterium is resistant to these detrimental conditions and whether its redox regulation has any role in dormancy is not studied yet.

1.6 Role of ROS as Signalling Molecules

Although previously thought to be purely harmful, recent studies have highlighted the significant role of ROS as physiological regulators of intracellular signalling pathways [55]. ROS mediated signalling is controlled by a delicate balance between its formation and scavenging [56]. The thiol functional group of cysteine are especially sensitive redox targets, oxidative modifications of which can lead to various downstream effects that modulate cell physiology [57, 58]. Superoxide and hydrogen peroxide are involved in regulation of cellular functions in various mammals and plants. For example the response of rat vascular smooth muscle cells to platelet-derived growth factor that includes tyrosine phosphorylation, mitogen activated protein kinase stimulation, DNA synthesis and chemotaxis, was inhibited when H_2O_2 was blocked [59]. Studies have reported that ROS are involved in the intracellular transduction of Ang-II signals for immediate early genes induction, cell proliferation and hypertrophic response [60]. In plants, recently it has been shown that ROS plays an important role in various cellular pathways such as growth, development, response to abiotic and biotic environmental stimuli and programmed cell death [56]. Moreover, these reactive oxygen species can specifically be produced and amplified by various cells for signalling purpose. Even in lower eukaryotes like Dictyostelium, superoxide signalling was found to enable the transition from unicellular to multi-cellular phase [61]. The most well studied signalling systems in prokaryotes are the OxyR and SoxR systems of *Escherichia coli*. These systems are activated by free radicals to combat the stress caused by free radicals. Hydrogen peroxide directly oxidizes OxyR protein to form an intramolecular disulphide bond which activates transcription factors to combat peroxide stress and superoxide activates the SoxR protein by oxidising its Fe/S centre [62, 63].

Thus ROS as signalling molecules in eukaryotes as well as prokaryotes have been shown to be key physiological regulators of many cellular functions, such as transcriptional regulation, direct oxidative modification, protein turnover, protein-protein interaction, and enzyme modification. However, the importance of ROS generation in mycobacteria has never received due acknowledgement.

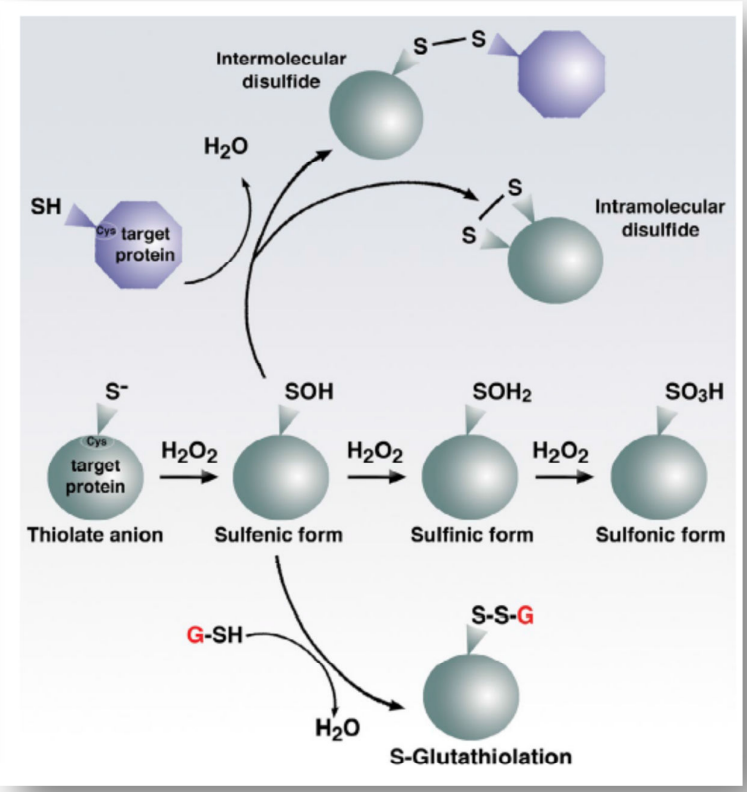


Figure 1.7: Redox dependent signalling through cysteine modification. Extracted from JCB, Review, 194, 2011.

1.7 Latency

1.7.1 Characteristics of Latency

Bacterial persistence is a hallmark of tuberculosis (TB). The ability of Mtb to programme itself into entering long periods of latency makes it a successful pathogen [64, 65]. Latency can be defined as "any tuberculosis lesion which fails to produce symptoms of its presence" [66]. Despite the immune system's ability to clear much of the bacilli and arrest an infection, the lungs may still contain small caseous foci. The first evidence of latent TB in the caseous foci was the reoccurrence of an infection

with non drug resistant tubercle bacilli after treatment with a regimen of antibiotics [67]. The development of antibiotic resistance in non-replicating dormant bacteria which is described as 'phenotypic drug-resistance' or 'drug-tolerance' is due to changes in the physiological state of the bacteria and not conferred by any inheritable genetic resistance mechanism [65]. There is an urgent need to study the metabolic processes that are critical for the pathogen to go into dormancy, survive under this non-replicating drug-resistant state, and get reactivated when the immune system of the host.

Latency is achieved by cell-mediated immune response which restricts the growth of *M. tuberculosis* bacilli. The restriction, however, does not eliminate the pathogen, leaving the bacilli as a present danger to reactivate years later [68]. Individuals infected with *M. tuberculosis* have a 10% chance during their life time to develop active tuberculosis from a latent infection. 5% of the infected population will develop the disease after 5 years and the others will suffer from it at some point during their lives [69]. Often, the precipitating factor for latent tuberculosis reactivation is a declining immunity, which takes place mostly in the elderly at an estimated rate of 5% per year until complete disappearance of immunity [70]. Factors such as corticosteroids, immunosuppressant, HIV and other factors that lower resistance are a danger for reactivation as well.

1.7.2 How to study Latency?

Treatment of latent infection with *M. tuberculosis* has been started quite early subsequent to the re-discovery of isoniazid in 1951 [71]. But, still there are many problems associated with it like long duration of treatment and side effects of the drugs [72]. Therefore, there is an urgent need to develop new drugs that can specifically kill the latent form and shorten the duration of treatment. To develop new drugs against the latent state of mycobacteria, one should know the precise nature of these dormant forms. A variety of factors are produced in granulomas to limit the bacterial growth. This includes oxygen and nutrient deprivation, acidic pH, and production of host factors such as nitric oxide [65]. Based on this, different dormancy models have been developed for better understanding of the mechanisms of dormancy and resuscitation. The following diagram shows evolution of different dormancy models with time [64].

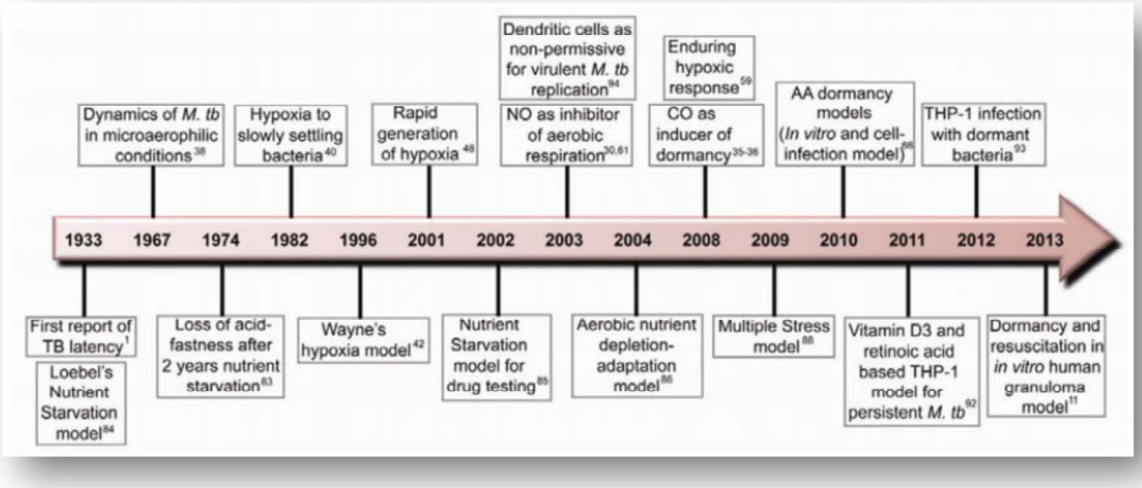


Figure 1.8: Evolution of Dormancy Models. The arrow is not drawn to the scale. Extracted from Current Science, 105, 2013.

1.7.3 Models to study Latency

1.7.3.1 In vitro Models

1.7.3.1.1Wayne Model

Several studies have been carried out to understand the *in vitro* sustainability of *M. tuberculosis* in closed necrotic lesion. Limited amounts of bacilli have been shown to survive twelve year incubation at 37°C in a sealed culture vessel, suggesting the capability of long term persistence in nutrient limited or anaerobic environment [73]. In the Wayne model, a sealed, standing culture is allowed to incubate over a period of days. In this condition, the bacteria deplete the available oxygen and thus anaerobic conditions are created [74, 75]. Addition of gentle stirring and a defined culture-to-headspace ratio were found to improve reproducibility [76]. Two distinct states of non-replicating persistence (NRP stage 1 and 2) are evident. These stages reflect discrete metabolic and drug susceptibility states compared with log phase growth. The first stage designated NRP-1 (non-replicating persistence stage 1), occurred when the declining oxygen level reached 1% saturation (equivalent to microaerophilic conditions) and this stage is characterized by increased production of glycine dehydrogenase and steady ATP generation. The second stage, NRP 2, happened when the oxygen level reached 0.06% saturation (equivalent to anaerobic

conditions) and this stage is characterized by a marked decline of glycine dehydrogenase and susceptibility to metronidazole [76]. The protein synthesis was down regulated but bacteria remained viable [77-79]. The bacilli in NRP 1 or NRP2 stage of the Wayne model gave about 10^8 cfu/ml [76], presumably because the relatively short time 10-14 days employed is not sufficient to convert most bacilli to dormant stage yet. According to the definition of dormancy (which refers to bacteria unable to form colonies on direct plating but can be resuscitated under appropriate conditions), the Wayne TB "dormancy" model does not fit this criterion and appears to be more like a model of low oxygen adaptation. Nevertheless, it has led to the identification of several factors that are related to persistence *in vivo* [80, 81]. Thus the Wayne model may represent a stage on the way towards dormancy and would still be a useful model to study dormancy as low oxygen may trigger dormancy upon extended incubation. It was also postulated that Wayne model bacteria may not represent the in vivo Mtb bacteria [82, 83].

1.7.3.1.2 Nutrient starvation Model

In granulomas, the environment is believed to be deprived of nutrients and essential cofactors [84-86]. Several studies till now have shown that Mtb bacilli can remain viable in distilled water and PBS for prolonged periods of time [87, 88]. Based on these findings, a nutrient starvation model (also referred to as the Loebel model) was developed to characterize the physiology of Mtb under dormant conditions [89]. In this model, Mtb bacilli are transferred from nutrient rich medium to nutrient poor medium such as PBS. Bacteria remain viable and static under such conditions for 6 week period. Resistance to standard drugs, unusual colony morphology and staining property, reduced respiration and bacteriostasis shows that the nutrient starved bacilli are in a dormant state [64].

1.7.3.1.4 Multiple Stress Dormancy Model

An in vitro multiple stress model was developed to more closely mimic the scenario that *M. tuberculosis* encounters within the host [90]. In this model bacteria were exposed to multiple stresses of low oxygen (5%), high CO_2 (10%), low carbon and nitrogen nutrients and acidic pH (5.0). In response to these stresses, *M. tuberculosis* stopped replicating, lost acid-fastness, became tolerant to anti-tubercular agents like rifampicin and isoniazid and accumulated wax esters and triglycerides.

Many genes which are expressed in Wayne model and nutrient starvation model were found to be expressed in this model also [91, 92].

1.7.3.2 Cell Infection Models

1.7.3.2.1 Vitamin C Induced Dormancy Model

Although *in vitro* models are available, they do not reflect the exact picture of what is happening in *in vivo* condition. *Ex vivo* models are now developed which have advantages over both *in vitro* as well as *in vivo* models [64, 93]. The 'vitamin C or ascorbic acid (AA)-dormancy infection model' over-comes the limitations of existing intracellular models [94]. In this model, THP-1 cells are exposed to vitamin C post-infection with *M. tuberculosis.* Resistance to isoniazid and induction of DevR dormancy regulon shows that the bacteria have become dormant. The transfer of vitamin C-exposed cultures to vitamin-free media restored growth properties and Isoniazid sensitivity. Thus, this model offers an alternative to other models of non-replicating persistence of *M. tuberculosis.*

1.7.3.2.2 Macrophage Infection Model

A model is developed by infecting THP-1 cells with non-replicating NRP-2 tubercle bacilli generated using the Wayne model of dormancy [95]. Phagosomes containing dormant bacilli were lysed. Dormancy related genes were induced. They thus described a model which can help understand how dormant bacilli survive intra-cellularly and influence the maintenance of hypoxic granuloma [64].

1.7.3.3 *In vivo* models

It is very important to have an ideal *in vivo* model of latent TB that can replicate the actual human latency to study the host-parasite interaction, the activity of drugs, and mechanism of latency. It is important to develop and establish a new animal model and/or improves an existing model for evaluating anti-tuberculosis activity of novel and existing drugs. There are some common animal models such as mouse, guinea pig, rabbit, non-human primate (NHP), and zebra fish. Though there is not a single ideal model, still these models are used for studying *in vivo* activity of potential drugs [96].

Thus, latent Tb infection models have enormous significance in the studying the action of the molecules/drugs, mechanism of latency, and role of host immune responses. Although many models exist for studying latent TB infection *in vitro* and *in vivo*, attempts are needed in the direction of building an ideal model to elucidate those unresolved questions in a field of latent TB. All these models have their inherent merits and demerits. However, all of these models can be employed in conjunction to minimize their drawbacks. It is critical to evolve an ideal model which can mimic the true human conditions of latency.

1.8 Diagnosis of Tuberculosis

A complete medical evaluation for TB must include a medical history, a chest X-ray, and culture based examination.

1.8.1 Medical history. The medical history includes obtaining the symptoms of pulmonary TB: productive, prolonged cough of three or more weeks, chest pain, and hemoptysis, fever, chills, night sweats, appetite loss, weight loss, and easy fatigability [97]. If a persistent respiratory illness does not respond to regular antibiotics then there are greater chances that the individual is having TB.

1.8.2 Chest X-ray. Presence of cavities in the lungs is detected by X-rays (Figure 1.9) [98]. However, lesions may appear anywhere in the lungs. In disseminated TB a pattern of many tiny nodules throughout the lung fields is common - the so called miliary TB. In HIV and other immunosuppressed persons, any abnormality may indicate TB or the chest X-ray may even appear entirely normal. Abnormalities on chest radiographs may be suggestive of, but are never diagnostic of, TB. However, chest radiographs may be used to rule out the possibility of pulmonary TB in a person who has a positive reaction to the tuberculin skin test and no symptoms of disease.

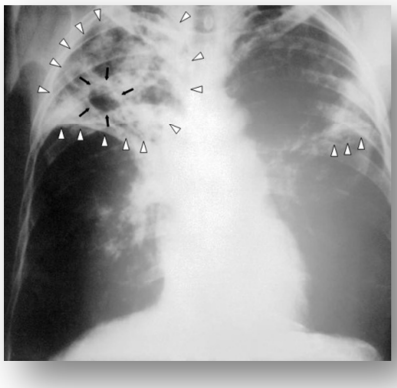


Figure 1.9: Chest X-ray of a person with advanced tuberculosis: Infection in both lungs is marked by white arrow-heads, and the formation of a cavity is marked by black arrows. Extracted from: http://en.wikipedia.org/wiki/Tuberculosis.

1.8.3 Microbiological Methods

1.8.3.1 Growth on selective media

The gold standard of TB diagnostics is confirmation with its growth on selective media [98]. This culturing is 1000 times more sensitive than microscopy, allows precise species identification, can be applied to drug susceptibility testing and may be useful to identify epidemiological links between patients or to detect laboratory cross contamination. In general, the sensitivity and specificity of culture method is 80-85% and 98% respectively [99]. However, their outcome is delayed by extremely low growth rate of mycobacteria. Contrary to a number of environmental mycobacteria that are rapid growers, yielding colonies in 7 days or less, *M. tuberculosis* exhibits a slow growth rate, requiring 14-21 days to generate visible colonies and does not produce any pigment (Fig. 1.9). With the advancement in culture system in 1980s BACTEC and biphasic culture methods were developed for faster recovery than traditional culture system [100].

Figure 1.10: *Mycobacterium tuberculosis* colonies on agar. Extracted from Centre for Tuberculosis Research, Johns Hopkins University.

1.8.3.2 Sputum testing for Acid fast bacilli

Acid fast staining of sputum samples should be done for detection of acid fast bacilli [97]. It is generally done byfluorescence microscopy (auramine-rhodamine staining), which is more sensitive than conventional Ziehl-Neelsen's staining [101, 102].

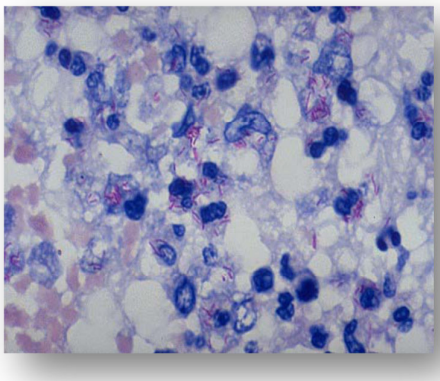


Figure 1.11: Acid fast staining of sputum containing *M. tuberculosis*. The bacteria are visible as small pink stained rods. Extracted from http://homepage.smc.edu/wissmann_paul/anatomy2textbook/histopathologypage1.html

1.8.3.3PCR

The introduction of nucleic acid amplification assays using polymerase chain reaction (PCR) in 1985 brought the most progress in TB diagnostics [103]. In 1989, PCR was first applied to clinical samples, sputum, gastric aspirates, abscess aspirates and biopsy samples to detect a mycobacterial gene which allows the differential diagnosis of *M. tuberculosis* from nontuberculous mycobacteria. A number of

candidate genes have been tested for usage as diagnostic targets. Among them, IS6110 repeat sequence has been used to detect *M. tuberculosis* directly in clinical samples [104]. However, some other factors such as an endogenous amplification inhibition factor of *M. tuberculosis* or unreliable quality control can influence susceptibility to both false positives and negatives and have hampered clinical uses of this assay [105]. However, the severity of problems began to reduce after development of automated, robust, commercial tests which made clinical risk assessment possible [106].

1.8.3.4Tuberculin Skin Test

Another old diagnostic method is the tuberculin skin test developed by Dr. Koch [107]. The test is for the identification of the host infected with *M. tuberculosis*. Tuberculin skin tests involve the intracutaneous injection of five tuberculin units of Purified Protein Derivative (PPD) prepared by the Montoux technique (Figure 1.12). The skin reaction of *M. tuberculosis* infection may discriminate from *M. avium* or other non-tuberculous infections [108]. However, the cross reactivity of PPD with *M. bovis* BCG vaccinated individuals represents a major limitation in applying this method to TB patients [109]. Therefore, the interpretation of the skin test needs to be made in the clinical context and with evaluation of other risk factors for infection.

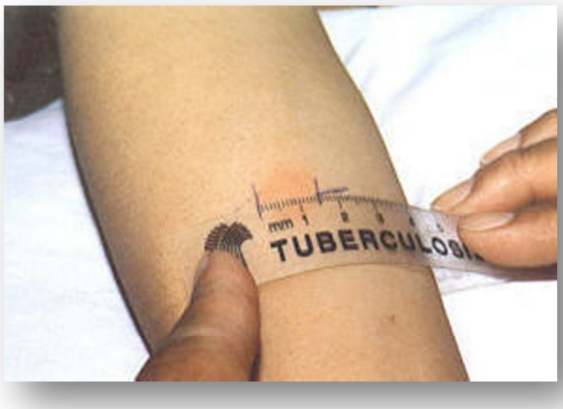


Figure 1.12: Tuberculin test. Extracted from http://drug-resistant-tb-fund.org/Eng/What%20is%20TB_Diagnosis.html

The identification of regions of the *M. tuberculosis* genome those are not present in *M. bovis* BCG and non tuberculous mycobacteria provide a unique opportunity to develop new specific diagnostic reagents. Fortunately, genomic studies

have shown that the region of differentiation (RD-1) is shared only by *M. tuberculosis*, *M. szulgai, M. marinum* and *M. kansasii*. The RD-1region contains the early secretary antigen target-6 (ESAT-6) and the culture filtrate protein-10 (CFP-10) that are potential targets of the specific immune response against *M. tuberculosis* [110]. Interferon (IFN)-γ secreted by TB patient's memory and effecter T-cells by the response of these proteins or overlapping peptides is a novel diagnostic marker of TB infection and could be discriminated from BCG vaccination or non-tuberculous mycobacteria infection [111]. In vitro blood test measuring IFN-γ is very useful to identify contacts of TB cases and show remarkable concordance with the tuberculin skin test [112]. Accordingly, the blood test has shown a greater sensitivity than the tuberculin skin test in active TB of HIV co-infected subjects. The recent progress in TB serology is also represented by the multi-antigen test using purified antigens. The recent studies of an Enzyme Linked-Immuno Sorbent Assay (ELISA) test using the 38Kda antigen, lipoarabinomannan (a mycobacterial lipoglycan, LAM), MPT-64, and glutamine synthase could achieve 93% sensitivity and 76% specificity with combination of AFB microscopy, suggesting that in the appropriate epidemiological and laboratory context this test could be used to improve the performances of the AFB smear test alone [113]. However, serological tests alone do not appear to help diagnosis of sputum positive pulmonary TB [114].

1.9 Control &Treatment of Tuberculosis

1.9.1 Control: BCG Vaccine

The live, attenuated *Mycobacterium bovis* BCG (bacillus Calmette-Guérin) is used in many countries for vaccination. The vaccine is acknowledged universally as being less than ideal [115], but the US Health Services to abandon it as a vaccine during the 1950s, after 40 years of diminishing tuberculosis cases. The use of this vaccine is debatable [116, 117].

1.9.2 Treatment: Chemotherapy

First drug to be used against Tb was streptomycin followed by p-amino salicylic acid (PAS) [118]. With the discovery of isoniazid, the first oral drug in 1952, and rifampin in 1957, chemotherapy started gaining importance. The discovery of the two effective drugs with their application in the armamentarium of anti-tuberculosis

strategy in 1966 [119,120] accelerated investigation on reduction of tuberculosis treatment. In the middle of 1970s, comparative studies of different combinations of these drugs showed superior curing efficiency for patients [121]. Isoniazid and rifampin were considered as complete bactericidal drugs, being capable of killing bacteria in all environments, while streptomycin and Pyrazinamide were of TB Treatment.

The current recommended treatment regime for drug-susceptible TB consists of a course of rifampicin (RIF), isoniazid (INH) and Pyrazinamide (PZA) for a two month period, possibly augmented by ethambutol (EMB) or streptomycin. This is followed by a further four months of RIF and INH treatment. This multi-drug cocktail therapy is used in order to minimise development of drug resistance via the complementary sterilising action of the differing drugs. This is combined with the practice of directly observed treatment (DOT) for insurance of patient compliance to therapy, as non-adherence to complete antibiotic courses is thought to greatly facilitate the prevalence of drug resistant TB [122]. MDR-TB requires a more aggressive drug therapy regimen over a longer duration of time for best chance of eradication [123].

1.9.2.1 Mode of action of drugs

All these drugs were discovered more than 40 years back. TB can usually be cured with a combination of first-line drugs taken for several months. Shown here are the four drugs in the standard regimen of first-line drugs and their modes of action.

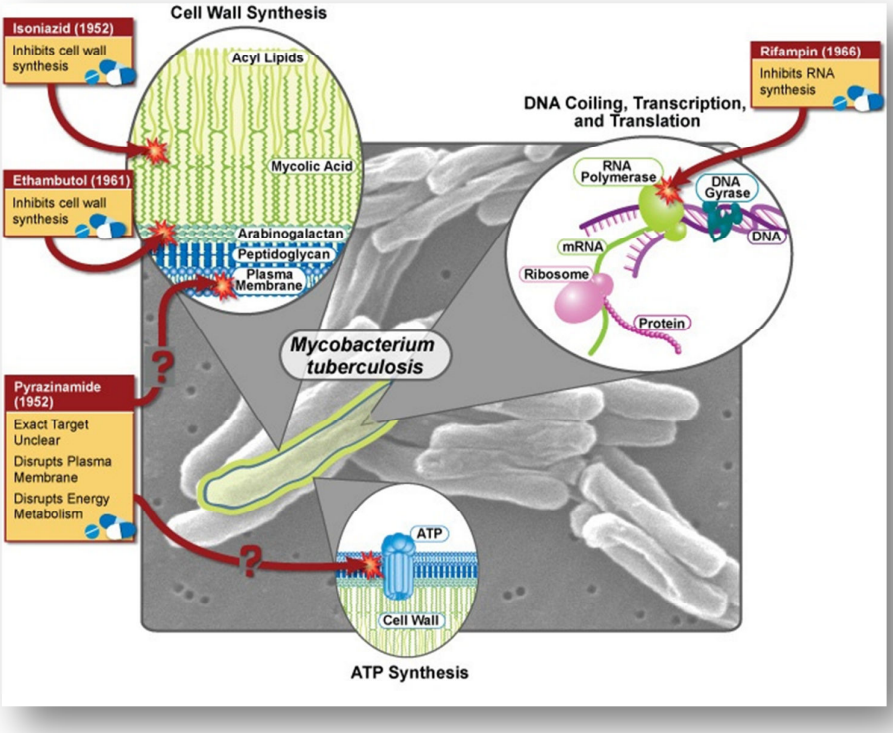


Figure 1.13: Mode of action of anti-tubercular drugs. Extracted from: Public domain of National Institute of Allergy and Infectious Diseases (NIAID)

According to their mode of action TB drugs can be grouped as inhibitors of cell wall biosynthesis (D-cycloserine, isoniazid, ethionamide and ethambutol), inhibitors of protein synthesis (streptomycin, kanamycin and capreomycin), inhibitors of nucleic acid synthesis (rifampin, quinolones) and inhibitors of membrane energy metabolism (Pyrazinamide) (Table 1.1) [124]. Their mode of action is described in the following Table 1.1

Table 1.1: Classes of anti-tubercular drugs and their mode of action

Ethambutol (ETB)	Inhibits mycobacterial arabinosyl transferases (encoded by the embCAB operon) involved in the polymerization of D-arabinofuranose to arabinoglycan, an essential cell wall component.	Ethambutol is generally bacteriostatic, but at high doses (25mg/kg) can be bactericidal (). Inhibits susceptible strains of *M. tuberculosis* at concentrations of 1–5μg/ml.
Streptomycin, kanamycin, amikacin, capreomycin	The aminoglycosides are irreversible inhibitors of protein synthesis through binding to specific 30S-subunit ribosomal proteins.	Bactericidal. *In vitro* and *in vivo* clinical data support use.
Ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gatifloxacin, sparfloxacin	Inhibit bacterial DNA synthesis through inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV, which are responsible for the relaxation of supercoiled DNA and the separation of replicated chromosomal DNA, respectively.	Bactericidal, broad spectrum antibacterials (). In-vitro and in-vivo clinical data support use (). Ciprofloxacin and levofloxacin inhibit strains of *M. tuberculosis* at concentrations of less than 2μg/ml. Newer agents (moxifloxacin, gatifloxacin, sparfloxacin) have lower minimum inhibitory concentrations.
Ethionamide	Chemically related to INH, converted via oxidation to ethionamide sulfoxide, blocks the synthesis of mycolic acids.	Inhibits most tubercle bacilli at concentrations of 2.5μg/mL or less.
Cycloserine	Structural analogue of D-alanine, inhibits incorporation of D-alanine into peptidoglycan pentapeptide through inhibition of alanine racemase.	Inhibits strains of *M. tuberculosis* at concentratins of 15–20μg/ml.
P-aminosalicylic acid (PASA)	Anti-metabolite interfering with incorporation of para-aminobenzoic acid into folic acid – folate synthesis antagonist.	Inhibits tubercle bacilli at concentrations of 1–5μg/ml.

Ethambutol (ETB)	Inhibits mycobacterial arabinosyl transferases (encoded by the embCAB operon) involved in the polymerization of D-arabinofuranose to arabinoglycan, an essential cell wall component.	Ethambutol is generally bacteriostatic, but at high doses (25mg/kg) can be bactericidal (). Inhibits susceptible strains of *M. tuberculosis* at concentrations of 1–5μg/ml.
Streptomycin, kanamycin, amikacin, capreomycin	The aminoglycosides are irreversible inhibitors of protein synthesis through binding to specific 30S-subunit ribosomal proteins.	Bactericidal. *In vitro* and *in vivo* clinical data support use.
Ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gatifloxacin, sparfloxacin	Inhibit bacterial DNA synthesis through inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV, which are responsible for the relaxation of supercoiled DNA and the separation of replicated chromosomal DNA, respectively.	Bactericidal, broad spectrum antibacterials (). In-vitro and in-vivo clinical data support use (). Ciprofloxacin and levofloxacin inhibit strains of *M. tuberculosis* at concentrations of less than 2μg/ml. Newer agents (moxifloxacin, gatifloxacin, sparfloxacin) have lower minimum inhibitory concentrations.
Ethionamide	Chemically related to INH, converted via oxidation to ethionamide sulfoxide, blocks the synthesis of mycolic acids.	Inhibits most tubercle bacilli at concentrations of 2.5μg/mL or less.
Cycloserine	Structural analogue of D-alanine, inhibits incorporation of D-alanine into peptidoglycan pentapeptide through inhibition of alanine racemase.	Inhibits strains of *M. tuberculosis* at concentratins of 15–20μg/ml.
P-aminosalicylic acid (PASA)	Anti-metabolite interfering with incorporation of para-aminobenzoic acid into folic acid – folate synthesis antagonist.	Inhibits tubercle bacilli at concentrations of 1–5μg/ml.

(Data extracted from Respiratory Research 2006 7:118)

1.10 Thesis Objectives

The major aim of this study was to understand how latency is achieved in *M. smegmatis*. Although there are different models to explain how mycobacteria become latent, but still none have explained the actual/central mechanism by which latency is achieved. In our study, we have tried to understand the mechanism of dormancy and resuscitation in *M. smegmatis*.

Lot of research is focussed on the generation of ROS by the host macrophages and strategies evolved by mycobacteria to overcome the oxidative stress. Recently, ROS has started gaining importance for being signalling molecules necessary for many cellular pathways. Also, there are reports which say that ROS generated by host actually helps mycobacteria to grow inside macrophages. All these studies prompted us to study role of ROS in mycobacteria. Till now, there are no reports of ROS generation by mycobacteria and its physiological effect on its growth.

In chapter 2, we have shown generation of total ROS by actively growing as well as dormant *M. smegmatis* bacilli. We observed more ROS in Wayne model of dormant culture than in aerobically growing culture. We have further extended our study to specifically measure superoxide in theses cultures by different colorimetric as well as flourimetric assays. We used different ROS modulators and inhibitors to determine the source of this superoxide. It was found that NADH oxidase is the major source of superoxide along with fumarate reductase being a secondary source.

As NADH oxidase was found as the major source of superoxide, we wanted to see the effect of inhibition of NADH oxidase on the growth of *M. smegmatis* cells. We observed that *M. smegmatis* cells become dormant and non-cultivable after inhibition by DPI which is an NADH oxidase inhibitor. Further characterization of this dormancy phenotype is done and we have developed a model using this inhibitor which can be used to make dormant *M. smegmatis* cells (Chapter 3). We have also shown resuscitation of these dormant cells by extraneous addition of superoxide which proves involvement of superoxide in the transition from active to dormant and vice a versa.

In our chapter 4, we have studied the changes taking place in these dormant bacilli at molecular level by microarray studies. We have also studied global

expression of proteins in active and dormant cells. It is very important to study dormancy at molecular and protein level because that can help us to identify different targets for the development of new drugs for these dormant bacilli which is a major problem in curing TB.

Overall, this study will help us to understand the mechanism by which latency and resuscitation is achieved. This understanding is very important in terms of the development of new drugs that can specifically kill these latent bacilli.

1.11 References

1. Smith Issar, Mycobacterium tuberculosis Pathogenesis and Molecular Determinants of Virulence, Clinical Microbiology Reviews, 16:463-496, 2003.

2. Hett E.C. and Rubin E.J., Bacterial Growth and Cell Division: a Mycobacterial Perspective, Microbiology and Molecular Biology Reviews, 72:126-156, 2008.

3. Ducati R.G., Ruffino-Netto A., Basso L.A. & Santos D.S, the Resumption of Consumption: A Review on Tuberculosis, Mem Inst Oswaldo Cruz, 101: 697-714, 2006.

4. Briken V., Porcelli S.A., Besra G.S. & Kremer L., Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response, Mol Microbiol 53: 391-403, 2004.

5. Vergne I., Chua J. & Deretic V., *Mycobacterium tuberculosis* phagosome maturation arrest: selective targeting of PI3P-dependent membrane trafficking, Traffic 4: 600-6, 2003.

6. Pathak S.K., Basu S., Bhattacharyya A., Pathak S., Kundu M. & Basu J., *Mycobacterium tuberculosis* lipoarabinomannan-mediated IRAK-M induction negatively regulates Toll-like receptor-dependent interleukin-12 p40 production in macrophages, J Biol Chem 280: 42794-800, 2005.

7. Nigou J., Zelle-Rieser C., Gilleron M., Thurnher M. & Puzo G., Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: evidence for a

negative signal delivered through the mannose receptor, J Immunol, 166: 7477-85, 2001.

8. Vergne I., Chua J., Lee H.H., Lucas M., Belisle J. & Deretic V., Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*, Proc Natl Acad Sci USA, 102: 4033-8. 2005. 9. Dao D.N., Kremer L., Guerardel Y., Molano A., Jacobs W.R., Porcelli S.A. & Briken V., *Mycobacterium tuberculosis* lipomannan induces apoptosis and interleukin-12 production in macrophages, Infect Immun, 72: 2067-74, 2004.

10. Ciaramella A., Martino A., Cicconi R., Colizzi V. & Fraziano M., Mycobacterial 19- kDa lipoprotein mediates *Mycobacterium tuberculosis* induced apoptosis in monocytes/macrophages at early stages of infection, Cell Death Differ, 7: 1270-2, 2000.

11. Means T.K., Wang S., Lien E., Yoshimura A., Golenbock D.T. & Fenton M.J., Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*, J Immunol, 163: 3920-7, 2002.

12. Ciaramella A., Cavone A., Santucci M.B., Garg S.K., Sanarico N., Bocchino M., Galati D., Martino A., Auricchio G., D'Orazio M., Stewart G.R., Neyrolles O., Young D.B., Colizzi V.& Fraziano M., Induction of apoptosis and release of interleukin-1 beta by cell wall-associated 19-kDa lipoprotein during the course of mycobacterial infection, J Infect Dis, 190: 1167-76, 2004.

13. Camus J.C., Pryor M.J., Medigue C. and Cole S.T., Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv, Microbiology, 148: 2967-2973, 2002.

14. Draper P. and Daffe M., The cell envelope of Mycobacterium tuberculosis with special reference to the capsule and outer permeability barrier in tuberculosis and tubercle bacillus. (Cole ST, Eisenach KD, Macmurray DN, Jacobs Jr. WR, Editors.) pp. 261-173. ASM press Washington DC.

15. Gordon R.E. and Smith M.M., Rapidly growing, acid fast bacteria. I. Species' descriptions of Mycobacterium phlei Lehmann and Neumann and *Mycobacterium*

smegmatis (Trevisan) Lehmann and Neumann, Journal of bacteriology, 66: 41–8, 1953.

16. Tsukamura M., Properties of *Mycobacterium smegmatis* freshly isolated from soil. Jpn J Microbiol20, 355-6, 1976.

17. Newton J.A., Jr. Weiss P.J., Bowler W.A. & Oldfield E.C., III. Soft-Tissue Infection Due to *Mycobacterium smegmatis*: Report of Two Cases. Clinical Infectious Diseases16, 531-533, 1993.

18. Gillespie S.H., Tuberculosis: evolution in millennia and minutes. Biochem Soc Trans35, 1317-20, 2007.

19. Anton V., Rouge P. and Daffe M. Identification of the sugars involved in mycobacterial cell aggregation. FEMS Microbiol Lett144, 167-70, 1996.

20.JCVI. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome&cmd=Retrieve&dopt=Overview&list_uids=20087, 2006.

20. Pérez E.,Pérez E., Gavigan J., Otal I., Guilhot C., Pelicic V., Giquel B. and Martin C.,Tn611 Transposon Mutagenesis in *Mycobacterium smegmatis* Using a Temperature-Sensitive Delivery System, 101: 187-198, 1998.

21. Chaturvedi V., Dwivedi N., Tripathi R.P. and Sinha S. Evaluation of *Mycobacterium smegmatis* as a possible surrogate screen for selecting molecules active against multi-drug resistant *Mycobacterium tuberculosis*, The Journal of General and Applied Microbiology53, 333-337, 2007.

22. Shiloh M.U. and DiGiuseppe Champion P.A. To catch a killer. What can mycobacterial models teach us about *Mycobacterium tuberculosis* pathogenesis? Current Opinion in Microbiology13, 86-92, 2010.

23. Kudykina Y.K., Shleeva M.O., Artsabanov V.Y., Suzina N.E. and Kaprelyants A.S. Generation of dormant forms by *Mycobacterium smegmatis* in the post stationary phase during gradual acidification of the medium, Microbiology80, 638-649, 2010.

24. Lee J.M., Cho H.Y., [...] and Oh J., O_2^- and NO-Sensing Mechanism through the DevSR Two-Component System in *Mycobacterium smegmatis*, Journal of Bacteriology190, 6795-6804, 2008.

25. WHO- Global Tuberculosis Report 2013 (apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf)

26. Smith Issar, *Mycobacterium tuberculosis* Pathogenesis and Molecular Determinants of Virulence, Clinical Microbiology Reviews, 16:463-496, 2003.

27. Bermudez L.E. and Goodman J., *Mycobacterium tuberculosis* invades and replicates within type II alveolar cells, Infect. Immun, 64:1400–1406, 1996.

28. Mehta P.K., King C.H., White E.H., Murtagh J.J., Jr. and Quinn F.D., Comparison of in vitro models for the study of *Mycobacterium tuberculosis* invasion and intracellular replication, Infect. Immun, 64:2673–2679, 1996.

29. Tascon R.E., Soares C.S, Ragno S., Stavropoulos E., Hirst E.M. and Colston M.J., *Mycobacterium tuberculosis*-activated dendritic cells induce protective immunity in mice, Immunology 99:473–480, 2000.

30. Bonder K.A., Serbina N.V. and Flynn J.L., Fate of *Mycobacterium tuberculosis* within murine dendritic cells, Infect. Immun, 69:800–809, 2001.

31. Gonzalez-Juarrero M. and Orme I.M., Characterization of murine lung dendritic cells infected with *Mycobacterium tuberculosis*, Infect. Immun, 69:1127–1133, 2001.

32. Schlesinger L.S. Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors, J. Immunol, 150:2920–2930, 1993.

33. Fenton M.J. and Vermeulen M.W., Immunopathology of tuberculosis: roles of macrophages and monocytes, Infect. Immun, 64:683–690, 1996.

34. Armstrong J.A. and Hart P.D.A., Phagosome-lysosome Interactions in cultured macrophages infected with virulent tubercle bacilli, J. Exp. Med, 142:1–16, 1975.

35. Frehel C., de Chastellier C., Lang T. and Rastogi N., Evidence for inhibition of fusion of lysosomal and prelysosomal compartments with phagosomes in

macrophages infected with pathogenic *Mycobacterium avium*, Infect. Immun, 52:252–262, 1986.

36. Sturgill-Koszycki S., Schlesinger P.H., Chakraborty P., Haddix P.L., Collins H.L., Fok A.K., Allen R.D., Gluck S.L., Heuser J. and Russell D., Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase, Science, 263:678–681, 1994.

37. Malik Z.A., Denning G.M. and Kusner D.J, Inhibition of Ca2_ signalling by Mycobacterium tuberculosis is associated with reduced phagosome-lysosome fusion and increased survival within human macrophages, J. Exp. Med, 191:287–302, 2000.

38. Noss E.H., Pai R.K., Sellati T.J., Radolf J.D., Belisle J., Golenbock D.T., Boom W.H. and Harding C.V., Toll-like receptor 2-dependentinhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of *Mycobacterium tuberculosis*, J. Immunol, 167:910–918, 2001.

39. Thoma-Uszynski S., Stenger S., Takeuchi O., Ochoa M.T., Engele M., Sieling P.A., Barnes P.F., Rollinghoff M., Bolcskei P.L., Wagner M., Akira S., Norgard M.W., Belisle J.T., Godowski P.J., Bloom B.R. and Modlin R.L., Induction of direct antimicrobial activity through mammalian toll-like receptors, Science, 291:1544–1547, 2001.

40. Via L.E., Deretic D., Ulmer R.J, Hibler N.S., Huber L.A. and Deretic V., Arrest of mycobacterial phagosome maturation is caused by a blocking vesicle fusion between stages controlled by rab5 and rab7, J. Biol. Chem, 272:13326–13331, 1997.

41. Ferrari G., Langen H., Naito M. and Pieters J., A coat protein on phagosomes involved in the intracellular survival of mycobacteria, Cell, 97:435–447, 1999.

42. Schuller S., Neefjes J., Ottenhoff T., Thole J. and Young D., Coronin is involved in uptake of *Mycobacterium bovis* BCG in human macrophages but not in phagosome maintenance, Cell, Microbiol, 3:785–793, 2001.

43. van Crevel R., Ottenhoff T.H. and van der Meer J.W., Innate immunity to *Mycobacterium tuberculosis*, Clin. Microbiol. Rev., 15:294–309, 2002.

44. Dannenberg A. M., Jr. and Rook J.A., Pathogenesis of pulmonary tuberculosis: interplay of tissue-damaging and macrophage-activating immune responses. Dual mechanisms that control bacillary multiplication, p. 459–483, 1994. In B. R. Bloom (ed.), Tuberculosis: pathogenesis, protection, and control. American Society for Microbiology, Washington, D.C.

45. Shi L., Sohaskey C.D., North R.J. and Gennaro M.L., Transcriptional characterization of the antioxidant response of *Mycobacterium tuberculosis* in vivo and during adaptation to hypoxia in vitro, Tuberculosis, 1-6, 2007.

46. Nathan C. and Shiloh M., Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens, PNAS, 97:8841-8848, 2000.

47. Bryk R., Lima C.D., Erdjument-Bromage H., Tempst P. and Nathan C., Metabolic Enzymes of Mycobacteria Linked to Antioxidant Defense by a Thioredoxin-Like Protein, Science, 295:1073-1077, 2002.

48. Sareen D., Newton G.L., Fahey R.C. and Buchmeier N.A., Mycothiol is essential for growth of *Mycobacterium tuberculosis* Erdman, j. of Bacteriol. 185:6736–6740, 2003.

49. Rawat M., Newton G.L., Ko M., Martinez G.J., Fahey R.C. and Av-Gay Y., Mycothiol-Deficient *Mycobacterium smegmatis* mutants are hypersensitive to alkylating agents, Free Radicals and Antibiotics, Antimicrob. Agents and Chemotherapy, 46: 3348–3355, 2002.

50. Meylan P.R., Richman D.D. and Kornbluth R.S. Reduced intracellular growth of mycobacteria in human macrophages cultivated at physiologic oxygen pressure, Am. Rev. Respir. Dis, 145:947–953, 1992.

51. N. Hasan, N. Yusuf, Z. Toossi and N. Islam, Suppression of Mycobacterium tuberculosis induced reactive oxygen species (ROS) and TNF-α mRNA expression in human monocytes by allicin, FEBS Lett., 580: 2517–2522, 2006.

52. Oberley-Deegan R.E., Rebits B.W., Weaver M.R., Tollefson A.K., Bai X., McGibney M., Ovrutsky A.R., Chan E.D. and Crapo J.D., An oxidative environment

promotes growth of *Mycobacterium abscessus*, Free Radical Biology & Medicine 49:1666–1673, 2010.

53. Relay et al, Marcia A. Firmani and Lee W. Riley, Reactive Nitrogen Intermediates Have a Bacteriostatic Effect on *Mycobacterium tuberculosis* In Vitro, Journal of Clinical Microbiology, 40: 3162-3166, 2002.

54. Taneja N.K., Dhingra S., Mittal A., Naresh M. and Tyagi J.S., *Mycobacterium tuberculosis* Transcriptional Adaptation, Growth Arrest and Dormancy Phenotype Development Is Triggered by Vitamin C, PLoS ONE 5:e10860, 2010.

55. Finkel T., Signal transduction by reactive oxygen species, JCB, 7-15, 2011.

56. Bailey-Serres J. and Mittler R., The Roles of Reactive Oxygen Species in Plant Cells, Plant Physiology, 141:311, 2006.

57. Rainwater R., Parks D., Anderson M.E., Tegtmeyer P. and Mann K., Role of cysteine residues in regulation of p53 function, Mol. Cell. Biol., 15:3892, 1995.

58. Namgaladze D., Hofer H.W. and Ullrich V., Redox Control of Calcineurin by Targeting the Binuclear $Fe2^{+}$-$Zn2^{+}$Center at the Enzyme Active Site, The Journal of Biological Chemistry, 277:5962-5969, 2002.

59. Sundaresan M., Yu Z., Ferrans V-J, Irani K. and Finkel T., Requirement for Generation of H_20_2 for Platelet Derived Growth factor Signal Transduction, Science, 270:296-299, 1995.

60. Puri P.L., Avantagiatti M.L., Burgio V.L., Chirillo P., Collepardo D., Natoli G., Balsano C. and Levrero M., Reactive Oxygen Intermediates Mediate Angiotensin II-induced c-Jun•c-Fos Heterodimer DNA Binding Activity and Proliferative Hypertrophic Responses in Myogenic Cells, The journal of Biological chemistry, 270:22129-22134, 1995.

61. Bloomfield G. and Pears C., Superoxide signalling required for multicellular development of Dictyostelium, Journal of Cell Science, 116:3387-3397, 2003.

62. Zheng M., Aslund F. and Storz G., Activation of the OxyR Transcription Factor by Reversible Disulfide Bond Formation, Science, 279:1718, 1998.

63. Hidalgo E., Ding H. and Demple B., Redox signal transduction via iron-sulfur clusters in the SoxR transcription activator, TIBS, 22:207-210, 1997.

64. Sikri K. and Tyagi J.S., The evolution of Mycobacterium tuberculosis dormancy models, Current Science, 105:607-616, 2013.

65. Deb C., Lee C., Dubey V.S., Daniel J., Abomoelak B., Sirakova T.D., Pawar S., Rogers L. and Kolattukudy P.E., A Novel In Vitro Multiple-Stress Dormancy Model for *Mycobacterium tuberculosis* Generates a Lipid-Loaded, Drug-Tolerant, Dormant Pathogen, PLoS ONE, 4:e6077, 2009.

66. Amberson J.B., The significance of latent forms of tuberculosis, N. Engl. J. Med., 219: 572-576, 1938.

67. Fox W., Ellard G.A. and Mitchison D.A., Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications, Int. J. Tuberc. Lung. Dis, 3: S231–S279, 1999.

68. Lucas S.B., Histopathology in Clinical Tuberculosis. Davies PDO, Editor. Chapman and Hall: London, pp. 113-127, 1998.

69. Grange J.M., Immunophysiology and immunopathology of tuberculosis, in Clinical Tuberculosis. Davies PDO, Editor. Chapman and Hall: London, pp. 129-152, 1998.

70. Stead W.W and Lofgren J.P., Does the risk of tuberculosis increase with old age?, J. Infect. Dis, 147: 951-955, 1983.

71. Ferebee S.H., Mount F.W. and Palmer C.E., Tuberculosis prophylaxis trials in review, Public Health Rep, 72:703–704, 1957.

72. Leung C.C., Rieder H.L., Lange C. and Yew W.W., Treatment of latent infection with Mycobacterium tuberculosis: update 2010, Eur Respir J, 37:690-711, 2011.

73. Corper HJ and Cohn ML., The viability and virulence of old cultures of tubercle bacilli. Am. Rev. Tuberc., 28: 856-874, 1933.

74. Wayne L.G., Synchronized replication of *Mycobacterium tuberculosis*, Infect Immun, 17: 528–530, 1977.

75. Wayne L.G., and Lin K.Y., Glyoxylate metabolism and adaptation of *Mycobacterium tuberculosis* to survival under anaerobic conditions, Infect. Immun, 37: 1042–1049, 1982.

76. Wayne L.G. and Hayes L.G., An in vitro model for sequential study of shift down of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence, Infect Immun, 64: 2062–2069, 1996.

77. Wayne L.G. and Hayes L.G., Nitrate reduction as a marker for hypoxic shift down of *Mycobacterium tuberculosis*, Tuberc. Lung Dis.,79:127-132, 1998.

78. Hu Y.M., Butcher P.D., Sole K., Mitchison D.A. and Coates A.R., Protein synthesis is shutdown in dormant *Mycobacterium tuberculosis* and is reversed by oxygen or heat shock, FEMS Microbiol. Lett. 158:139-145, 1998.

79. Hu Y., Mangan J.A., Dhillon J., Sole K.M., Mitchison D.A., Butcher P.D. and Coates A.R., Detection of mRNA transcripts and active transcription in persistent *Mycobacterium tuberculosis* induced by exposure to rifampin or Pyrazinamide, J. Bacteriol., 182, 6358-6365, 2000.

80. McKinney J.D., Höner Zu Bentrup K., Munoz- Elias E.J., Miczak A., Chen B., Chan W.T., Swenson D., Sacchettini J.C., Jacobs W.R. Jr. and Russell D.G., Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase, Nature, 406:735-738, 2000.

81. Hong P.C., Tsolis R.M. and Ficht T.A., Identification of genes required for chronic persistence of Brucella abortus in mice, Infect. Immun. 68:4102-4107, 2000.

82. Dhillon J., Allen B.W., Hu Y.M., Coates A.R. and Mitchison D.A., Metronidazole has no antibacterial effect in Cornell model murine tuberculosis, Int. J. Tuberc. Lung Dis., 2:736-742, 1998.

83. Brooks J.V., Furney S.K. and Orme I.M., Metronidazole therapy in mice infected with tuberculosis, Antimicrob. Agents Chemother., 43:1285-1288 (1999)

84. Gomez, J. E. and McKinney, J. D., M. tuberculosis persistence, latency, and drug tolerance. Tuberculosis (Edina.), 2004, 84, 29–44.

85. Wayne, L. G., Dormancy of *Mycobacterium tuberculosis* and latency of disease. Eur. J. Clin. Microbiol. Infect. Dis., 1994, 13, 908–914.

86. Dietrich, J. and Doherty, T. M., Interaction of *Mycobacterium tuberculosis* with the host: consequences for vaccine development. Apmis, 2009, 117, 440–457.

87. Nyka, W., Studies on the effect of starvation on mycobacteria. Infect. Immun., 1974, 9, 843–850.

88. Loebel, R. O., Shorr, E. and Richardson, H. B., The influence of adverse conditions upon respiratory metabolism and growth of human tubercle bacilli. J. Bacteriol., 1933, 26, 167–200.

89. Betts, J. C., Lukey, P. T., Robb, L. C., McAdam, R. A. and Dun-can, K., Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. Mol. Microbiol., 2002, 43, 717–731.

90. Deb, C. et al., A novel in vitro multiple-stress dormancy model for *Mycobacterium tuberculosis* generates a lipid-loaded, drug-tolerant, dormant pathogen. PLoS One, 2009, 4, e6077.

91. Low, K. L., Rao, P. S., Shui, G., Bendt, A. K., Pethe, K., Dick, T. and Wenk, M. R., Triacylglycerol utilization is required for re-growth of in vitro hypoxic nonreplicating *Mycobacterium bovis* bacillus Calmette-Guerin. J. Bacteriol., 2009, 191, 5037–5043.

92. Singh, A., Gupta, R., Vishwakarma, R. A., Narayanan, P. R., Paramasivan, C. N., Ramanathan, V. D. and Tyagi, A. K., Requirement of the mymA operon for appropriate cell wall ultra-structure and persistence of *Mycobacterium tuberculosis* in the spleens of guinea pigs. J. Bacteriol., 2005, 187, 4173–4186.

93. Vergne, I., Chua, J., Singh, S. B. and Deretic, V., Cell biology of *Mycobacterium tuberculosis* phagosome. Annu. Rev. Cell. Dev. Biol., 2004, 20, 367–394.

94. Taneja, N. K., Dhingra, S., Mittal, A., Naresh, M. and Tyagi, J. S., *Mycobacterium tuberculosis* transcriptional adaptation, growth arrest and dormancy phenotype development is triggered by vitamin C. PLoS One, 2010, 5, e10860.

95. Iona, E. et al., Infection of human THP-1 cells with dormant *Mycobacterium tuberculosis.* Microbes Infect., 2012, 14, 959–967.

96. Patel K., Jhamb S.S. and Singh P.P., Models of Latent Tuberculosis: Their Salient Features, Limitations, and Development, J Lab Physicians, 3:75-79, 2011.

97. Kumar V., Abbas A.K., Fausto N. and Mitchell R.N., Robbins Basic Pathology (8^{th} edition), Saunders Elsevier. pp. 516-522, 2007.

98. Golyshevskaia V.I., Korneev A.A. and Chernousova L.N., New microbiological techniques in diagnosis of tuberculosis, Probl.Tuberk, 6: 22-25, 1996.

99. Steingart K., Henry M., Ng V., et al., Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review, Lancet Infect Dis, 6 (9): 570–81, 2006.

100. Brown M., Varia H., Bassett P., Davidson R.N., Wall R. and Pasvol G., Prospective study of sputum induction, gastric washing, and bronchoalveolar lavage for the diagnosis of pulmonary tuberculosis in patients who are unable to expectorate, Clin Infect Dis 44(11): 1415–20, 2007.

101. Morgan M.A., Horstmeier C.D., DeYoung D.R. and Roberts G.D. Comparison of radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear negative specimens, J. Clin. Microbiol., 18: 384-388, 1983.

102. Abe C., Hosojima S., Fukasawa Y., Kazumi Y., Takahashi M. and Hirano K., et al. Comparison of MB-check, BACTEC and egg based media for recovery of mycobacteria, J. Clin. Micobiol., 30: 878-881, 1992.

103. Brisson N.A., Gicquel B., Lecossier D., Levy-Frebault V., Nassif X. and Hance A.J. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples, Lancet, 2: 1069-1071, 1989.

104. Thiery D., Cave M.D., Isenach K.D., Crowford J.T., Bates J.H., Gicquel B., et al. IS 6110 an IS like element of *Mycobacterium tuberculosis* complex, Nucleic Acid Res, 18: 188, 1990.

105. Amicosante M., Richeldi L., Trenti G., Paone G., Campa M., Bisetti A., et al. Inactivation of polymerase inhibitors of *Mycobacterium tuberculosis* DNA amplification in sputum by using capture resin, J. Clin. Microbiol., 33: 629-630, 1995.

106. Jonas V., Alden M.J., Curry J.L., Kamisango K., Knott C.A., Lankford R., et al. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA, J. Clin. Microbiol., 31: 2410-2416, 1993.

107. Huebner R.E., Schein M.F., Bass J.B. Jr., The tuberculin skin test, Clin. Infect. Dis., 17: 968-75, 1993.

108. Palmer C.E. and Edwards L.B., The tuberculin test: in retrospect and prospect, Arch. Environ. Health, 15: 792-808, 1967.

109. Wang L., Turner M.O., Elwood R.K., Schulzer M. and FitzGerald J.M. A meta-analysis of the effect of bacille Calmette-Guérin vaccination on the tuberculin skin test measurements, Thorax, 57: 804–809, 2002.

110. Cockle P.J., Gordon S.V., Lalvani A., Buddle B.M., Hewinson R.G. and Vordermeier H.M. Identification of novel *Mycobacterium tuberculosis* antigens with potential as diagnostic reagents or subunit vaccine candidates by comparative genomics, Infect. Immun, 70: 6996-7003, 2002.

111. Goletti D., Carrara S., Vincenti D., Saltini C., Rizzi E.B., Schinina V., et al. Accuracy of an immune diagnostic assay based on RD1 selected epitopes for active tuberculosis in a clinical setting: a pilot study, Clin. Microbiol.Infect, 12: 544-550, 2006.

112. Pai M., Gokhale K., Joshi R., Dogra S., Kalantri S., Mendiratta D.K., et al. *Mycobacterium tuberculosis* infection in health care workers in rural India: comparison 37 of a whole-blood interferon gamma assay with tuberculin skin testing, JAMA, 293: 2746-2740, 2005.

113. Kanaujia G.V., Lam P.K., Perry S., Brusasca P.N., Catanzaro A. and Gennaro M.L. Integration of microscopy and serodiagnostic tests to screen for active tuberculosis, Int. J. Tuberc. Lung Dis, 9: 1120-1126, 2005.

114. Chan E.D., Heifets L. and Iseman M.D. Immunologic diagnosis of tuberculosis: a review, Tuber. Lung. Dis, 80: 131-140, 2000.

115. Fine P. E., Vaccines, genes and trials. Novartis Found, Symp, 217: 57–69, 1998.

116. Kaufmann S. H., Is the development of a new tuberculosis vaccine possible?, Nature Med., 6: 955–960, 2000.

117. Russel D.G., *Mycobacterium tuberculosis*: here today, and here tomorrow, Nature Reviews: Molecular Cell Biology, 2:1-7, 2001.

118. Schatz A., Elizabeth B. and Waksman S., Streptomycin: a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria, Proceedings of the Society for Experimental and Biological Medicine, 55: 66-69, 1944.

119. Gallo G.G., Magg N., Pasqualucc C.R., Ballott R. and Sens P., Rifamycins. XLII. Rifazine, a new phenazinic derivative of rifamycins, Farmaco Sci, 21:168-175, 1966.

120. Youatt J., A review of the action of isoniazid, Am. Rev. Respir. Dis., 5: 729-749, 1969.

121. Controlled trial of 4 short course (6 month) regimens of chemotherapy for treatment of pulmonary tuberculosis - Second trial, Am. Rev. Respir. Dis., 114: 471, 1976.

122. Cox H.S., Morrow M. & Deutschmann P.W., Long term efficacy of DOTS regimens for tuberculosis: systematic review, BMJ, 336:484-487, 2008.

123. Caminero J.A., Sotgiu G., Zumla A. and Migliori G.B., Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis, The Lancet Infectious Diseases, 10:621-629, 2010.

124. Zhang Y., The magic bullets and tuberculosis drug targets, Annu. Rev. Pharmacol. Toxicol, 45: 529-564, 2005.

CHAPTER 2

Comparison of Superoxide Levels in Active and Dormant *Mycobacterium smegmatis*

2.1 Introduction

Most studies till date have focused on defence mechanisms of mycobacteria to survive the oxidative pressure within macrophages [1] but none tried to explicate the role of oxidants in mycobacterial growth. Superoxide can spontaneously or enzymatically dismutate into hydrogen peroxide. SodA, an iron-dependent superoxide dismutase released into the extracellular medium was proposed to play an important role in detoxification of superoxide radicals [2]. Mtb also possess a copper-dependent superoxide dismutase (SodC) which is not essential for virulence of the organism in guinea pigs [3]. While, another report says that it is required for combating oxidative stress inside host macrophages [4]. Therefore, the role of SodC with respect to virulence and its growth inside host needs to be clearly established. Inside the phagosomes of activated macrophages different peroxides can be formed, including hydrogen peroxide, peroxynitrite and fatty acid hydroperoxides along with other oxidative species [5]. All of these have been reported to be cytotoxic against microorganisms including bacteria [1]. Mtb has evolved an elaborate mechanism to enzymatically detoxify these reactive oxygen and nitrogen species [6]. Mtb constitutively expresses aheme-dependent catalase peroxidase (KatG) which catalyses the conversion of hydrogen peroxide into harmless molecular oxygen. Interestingly, the Mtb mutant of KatG was avirulant as well as unable to survive during *in vitro* growth in culture medium [7]. Moreover, clinical isolates of a KatG mutant are found to over-express thiol-dependent alkyl hydroperoxidase C (AhpC), indicating the importance of both in ensuring protection of the pathogen against oxidizing species [8]. Mycothiol, a functional analogue of glutathione has been reported as essential for the growth of Mtb by protecting it from oxidative stress [9]. In *M. smegmatis* too, it was observed that mycothiol deficient mutant strains are more sensitive to nitric oxide and hydrogen peroxide mediated toxicity than the wild type cells [10]. ROS as signalling molecules in eukaryotes as well as prokaryotes has greatly been deliberated upon in the past decade. They have been shown to be key physiological regulators of many cellular functions, such as transcriptional regulation, direct oxidative modification, protein turnover, protein-protein interaction, and enzyme modification [11, 12]. However, the importance of investigating ROS generation in mycobacteria has never received due acknowledgement.

In this chapter, we have compared levels of total ROS and specifically superoxide in active as well as dormant *M. smegmatis.* One electron reduction of oxygen produces the superoxide anion which later on is converted to hydrogen peroxide and then to hydroxyl radical [13, 14]. Therefore it is very important to study superoxide production as it is the first radical to form among all species in ROS. We also show that its probable primary source in actively growing bacilli is the membrane associated NADH:flavinoxidoreductase (NADH oxidase).

The experimental disadvantages associated with the slow growth and infectious nature of *M. tuberculosis* prompted us to use *M. smegmatis* as a model as it showed strikingly similar behaviour to the characteristics of *M. bovis* BCG and *M. tuberculosis*in*in vitro* gradual oxygen depletion model as well as in nutrient starvation and non-culturable models [15-18].

2.2 Material and methods

2.2.1 Chemicals, Media and Strains

All chemicals were purchased from Sigma-Aldrich, USA except for Dubos medium, which was purchased from DIFCO, USA and 2-hydroxyethidium which was purchased from Noxygen, Germany. *M. smegmatis*strain *mc²155* was a gift from AstraZeneca, India. Sub-culturing of the strain was routinely performed on Dubos-albumin-agar slant. The stock was maintained at -70°C and sub-cultured once in liquid medium before inoculation in experimental culture medium. *M. smegmatis*culture was grown in 30 ml Dubos broth in a 100 ml flask incubated at 37°C on an orbital shaker (Thermo Electron Model No.131 481; Thermo Electron Corp., Marietta, OH) set at 150 rpm. All antioxidants, DCFH-DA and dihydroethidium (DHE) were freshly prepared in dimethylsulfoxide (DMSO) while fumarate, succinate and NADH were prepared in water.

2.2.2 Cultivation of oxygen depletion induced dormant bacilli

For the cultivation of anaerobic dormant bacilli, Wayne's 0.5 HSR tube model was followed [19]. 17.5 ml of medium was used throughout and, therefore, the ratio of head space air volume (8.5 ml) to liquid volume (17.5 ml) was always 0.5. Tightly sealed caps with rubber septa were used to permit addition of reagents by needle without opening the container. Media was inoculated with 1 % of 1 OD (620 nm) culture of *M. smegmatis* and incubated at 37°C at 120 rpm for 7 days. Dormancy was confirmed by CFU enumeration.

2.2.3 Cultivation of nutrient starvation induced dormant bacillli

The nutrient starvation induced dormant bacilli were obtained by following method provided by Betts and co-workers [20]. Briefly, *M. smegmatis*cells were grown under shaking conditions at 150rpm in 100ml *Dubos* medium up to 48 hours (late logarithmic phase). Cells were then harvested by centrifugation andwashed twice with phosphate-buffered saline (PBS). The washed cells were then transferred toflasks containing 100ml sterile PBS and incubated at 37°C and 150rpm shaking condition. After 10 days of incubation dormancy was confirmed by CFU enumeration [21].

2.2.4 Detection of Total ROS

The total ROS within *M.smegmatis* bacilli was measured by using 2', 7'-dichloro-dihydro-fluorescin-diacetate (DCFH-DA), a method developed earlier [22]. Freshly prepared DCFH-DA stock solution was added to aerobically growing culture of OD ~ 1.0 at 620 nm to a final concentration of 20µM. The incubation of the culture was carried out at 37°C in dark after which fluorescence was measured (excitation 485nm and emission 520nm) using a fluorescence spectrophotometer (LS 55, Perkin-Elmer, Foster City, CA). In case of Wayne model, the DCFH-DA solution was directly added into the Wayne tube on 7^{th} day, unless otherwise mentioned, through rubber septa using a syringe and fluorescence was measured after incubation. In case of nutrient starvation model, 10^{th} day culture was used.

To visualize the ROS generation by actively growing and dormant *M. smegmatis*, cells were centrifuged at 10,000 RPM for 10 minutes and washed by potassium phosphate buffer (20mM, pH 6.0) twice. Then cells were incubated with DCFH-DA at a final concentration of 20µM in PBS (20mM, pH 6.0) for 30 minutes at 37°C in dark. Again cells were washed twice with PBS as mentioned above. For Wayne model, the solution was directly added into the Wayne tube through rubber septa using a syringe and incubated for another 15 minutes. Microscopic images were captured using a fluorescence microscope (LeitzWetzlar, Germany) withI3 excitation filter (480nm) and Emission filter (520nm).

2.2.5 Assay for superoxide production using tetrazolium salt

Superoxide production by *M.smegmatis* bacilli was measured by using XTT; a method developed earlier [23].XTT (2, 3-bis- [2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide) stock solution (34mM) was prepared in PBS (pH 6.6) when required, or stored at -20°C.For aerobically growing bacilli, late log phase culture of 1.0 OD_{620} was washed with PBS (pH 6.6) and re-suspended in the same buffer containing 100µM XTT. It was then kept in dark at 37ºC inside a shaker incubator at 150rpm. After definite time interval, flask was taken out and the culture was centrifuged at 10,000rpm for 5 minutes. OD of supernatant was taken at λ_{470} because superoxide reacts with pale yellow XTT to form a bright orange-coloured formazan which can be measured at 470nm. In case of Wayne model, 50µl of the XTT solution was added with the help of a syringe to achieve a final concentration 100µM on 7^{th} day and then tubes were kept in dark at 37ºC at 130rpm. For nutrient

starvation model, XTT was added directly to the culture medium on 10^{th} day at a final concentration of 100µM, same protocol was then followed to take the absorbance. Media as well as solvent blank were also kept for normalization.

2.2.6 Detection of endogenous superoxide production by *Mycobacterium smegmatis*using DHE

Superoxide production by *M. smegmatis* was detected by following a modified HPLC-based method described earlier [24, 25]. Initially, 2 ml of 0.3 OD_{620} of aerobically growing *M. smegmatis* cells was taken washed by centrifugation at 10,000 rpm for 10 minutes at 4ºC and re-suspended in 1 ml of *M. Phlei* medium containing 100µM diethylenetriaminepentaacetic acid (DTPA). DHE stock solution was added to it to achieve a final concentration of 25µM, mixed well and incubated at 37ºC for 90 minutes. After incubation, Triton X100 was added to the cell suspension at a concentration of 8%, vortexed for 5 minutes followed by sonication in a water bath sonicator (Bandelin Electronics, Germany) at 35 kHz for 5 minutes. The cell lysate was filtered through 0.2µm membrane filter (Acrodisc 13 mm syringe filters, Pall Corporation) and the filtrate was taken for HPLC analysis (Waters). The chromatographic separation was performed on a C18 reverse phase column (Kinetex 5µ C18 100A, 250*4.60mm from Phenomenix). A gradient of solution A (1% formic acid in Water) and B (1% formic acid in ACN) was used as mobile phase at a flow rate of 0.4ml/min. Chromatographic runs were started with 100% solution A, decreased linearly to 60% solution A during the initial 10 minutes, continued at isocratic condition further for 15 minutes followed by a decrease to 0% solution A in the next 1minute, continued as 100% solution B for further 4 minutes. Solution A was then increased to 100% in next 1 minute continued this further for 9 minutes. 2-hydroxyethidium fluorescence was monitored by a fluorescence detector (excitation 480nm, emission 580nm) and DHE absorbance was monitored by a UV detector at 245nm. Area under the 2-hydroxyethidium peak was taken and concentration of 2-hydroxyethidium was calculated by extrapolation from standard graph.

To see the effect of ROS modulators on superoxide production by *M. smegmatis*, ROS modulators at their respective IC_{90} values were added to the above mentioned samples before addition of DHE and HPLC samples were prepared as above.

Superoxide production within the *M.smegmatis* bacilli could also be visualised by DHE fluorescent microscopy.*M. smegmatis* late log phase culture of 1 OD_{620} was washed with PBS and re-suspended in PBS containing 10μM DHE. These cells were incubated for two hours at 37°C on an orbital shaker at 150 rpm. Cell were washed with PBS and visualized by fluorescent microscopy (LeitzWetzlar, Germany) with I2 excitation filter (530nm) and Emission filter (600nm) under 400X magnification. For Wayne model, DHE was added through a syringe to 7^{th} day cultures. For nutrient starvation model, 10^{th} day cultures were used.

Detection of superoxide production by *Mycobacterium smegmatis*crude membrane was done. Briefly, 20μl of (0.8μg/ml of protein estimated by Bradford's method) membrane was taken in 1ml of *M. Phlei* medium containing 100 μM DTPA and 210μM NADH. 1μl of freshly prepared DPI was added at a final concentration of 10μg/ml. HPLC samples were prepared at different time points of incubation with DPI.

2.2.7 Crude membrane preparation of *Mycobacterium smegmatis*

M. smegmatis sphaeroplast were prepared by following an earlier described method [26]. Briefly, the method includes addition of sphaeroplast solution [cycloserine (0.006% w/v), lysozyme (0.002% w/v), lithium chloride (1% w/v) and Ethylenediaminetetraaceticacid(EDTA) (0.2% w/v)] to exponentially growing log-phase culture. It was then incubated for 12 hours at 37°C in a shaker incubator at 150 rpm. Sphaeroplast thus obtained were centrifuged at 10,000 rpm for 10 minutes and the pellet was re-suspended in *M. phlei* medium containing 100μM DTPA and then sonicated in a water bath sonicator at 35 kHzfor 5 minutes. The membrane fragments were then washed twice at 15,000 rpm for 30min at 4°C, re-suspended in the same medium and stored at -20°C. These membrane fragments were kept on ice before use.

2.2.8 Extraction protocol for quantification of NAD^+ and NADH redox states in active and dormant *M. smegmatis*

35 ml of 0.46 OD_{620} of aerobically growing cells (active) and 35 ml of 0.46 OD_{620} of 7^{th} day Wayne model culture cells (dormant) were taken and centrifuged at 10000rpm at 0°C for 10 minutes. Transfer of cells from Wayne tube to centrifuge tube was done on ice to cease all the metabolic activities. Pellet was re-suspended in 1 ml of distilled water containing 0.8% Triton X100, vigorously vortexed on a cyclomixer

for 5 minutes. After mixing, centrifugation was done at 10000rpm at 0°C for 5 minutes and supernatant was collected. 20 µl of chloroform was added to the 1ml supernatant, mixed and placed on ice to set for 30 minutes. After the separation of two layers, the aqueous layer was collected and filtered through 0.2µm syringe filter and stored at -20°C for HPLC analysis.

Chromatographic separation was performed by following a modified method described earlier [27]using a Hydrosphere C18 column (2.5 µm, 150 mm×4.6 mm id (Waters, Milford, MA, USA) at 25°C temperature. 30 µl aliquots of samples were used for injection. A gradient of solvent A (50 mM ammonium acetate) and solvent B (100% ACN) at flow rate of 1ml/min was used as a mobile phase for separation. Chromatographic runs were started with 100% solvent A followed by a decrease to 95% solvent A over 25 minutes. The column was washed after each separation by increasing mobile phase B to 90% for 7 min & equilibrated by 100% mobile phase A before sample inject. UV absorbance was monitored at 260 and 340 nm and pertinent peak areas integrated using area under the curve algorithms. Quantification of NAD^+ was assessed using absorbance at 260 nm. Since at 340 nm only NADH absorbs whereas NAD^+ does not, quantification of NADH was assessed using absorbance at 340 nm. Concentration of NAD^+ and NADH was calculated using a standard graph of NAD^+ and NADH.

2.3 Results

2.3.1 Comparison of Total ROS in active and dormant *Mycobacterium smegmatis* by DCFH-DA oxidation

One electron reduction of oxygen normally produces superoxide anion, a by-product of aerobic respiration [13]. Superoxide is converted to hydrogen peroxide by SOD present within a cell which is further converted to hydroxyl radical by the Fenton reaction. The term ROS includes superoxide radical, hydrogen peroxide and hydroxyl radical [14]. One of the very commonly used probes for detection of intracellular ROS is DCFH-DA [22].DCFH is oxidized by ROS to highly fluorescent 2', 7'-dichlorofluorescein (DCF) in a peroxidase dependent reaction. Although DCFH does not penetrate the plasma membrane, it can be introduced into the cell in the form of non-polar, un-reactive DCFH-diacetate that is subsequently deacetylated by endogenous esterase. As a result reactive DCFH is liberated intra-cellularly which in turn reacts with ROS to produce DCF and, remain trapped in the cytoplasm. DCFH can be oxidised by hydrogen peroxide, peroxynitrite, and lipid hydroperoxides and, to a lesser extent, superoxide. The percentage increase in fluorescence per well was calculated by formula $[(Ft–Ft_0)/ Ft_0*100]$, where Ft=fluorescence at time t and Ft_0 = Fluorescence at time 0 min.

The Percent relative fluorescence was found to be more in anaerobic culture than in nutrient starved and actively growing bacilli, indicating production of more reducing equivalents under anaerobic condition than in aerobic condition (Figure 2.1).

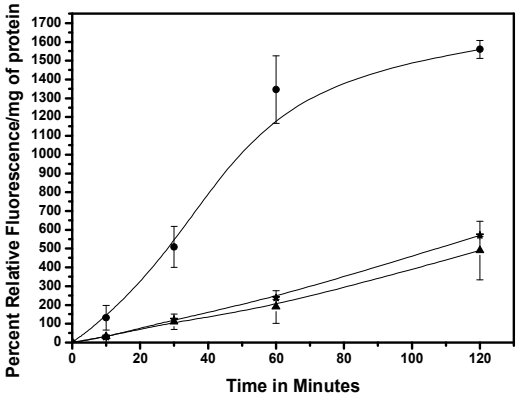


Figure 2.1: Comparison of ROS production by active and dormant *Mycobacterium smegmatis* cells. (■) 1 OD_{620} of aerobically growing *M. smegmatis*cells, (●) 7th day Wayne model culture and (▲) 10th day Nutrient starvation model culture were incubated with DCFH-DA (20µM) at 37°C and fluorescence measurements were taken at different time points using a fluorescence spectrophotometer as described in "Materials and Methods". The percent relative fluorescence per well was calculated by formula $[(Ft - Ft_0)/ Ft_0*100]$, where Ft = fluorescence at time t and Ft_0 = Fluorescence at time 0 min. All the experiments were performed in triplicates. Results are mean values with SD.

ROS production was also visualized by fluorescent microscopy using DCFH-DA probe. Fluorescent images of anaerobic, actively growing and nutrient starved cultures clearly shows production of ROS by active and dormant forms of *M. smegmatis* (Figure 2.2).

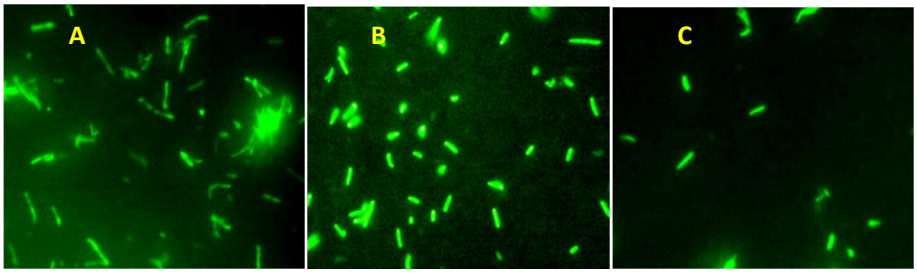


Figure 2.2: Detection of ROS by DCFH-DA oxidation using fluorescent microscopy in active and dormant cultures of *Mycobacterium smegmatis*A) Actively growing *M. smegmatis*cells B) 7th day Wayne model cells C) 10th day nutrient starved cells. Images are taken using a fluorescence microscope (LeitzWetzlar, Germany) withI3 excitation filter (480nm) and Emission filter (520nm) under 400X magnification.

2.3.2 Detection of superoxide by active and dormant *Mycobacterium smegmatis* cellsUsing XTT

XTT (Tetrazolium salt) is used widely for detecting the redox potential of cells and mainly superoxide in the cells [23, 28]. Reaction with superoxide causes the pale yellow XTT to form its bright orange-coloured formazan, the accumulation of which can be measured by monitoring its absorbance at 470 nm. XTT reduction in dormant culture was found to be faster than in active culture. In case of dormant culture 0.6 OD_{470} is achieved in 250 minutes whereas in same incubation time in aerobic culture, almost negligible OD_{470} is achieved (Figure 2.3). At least, the results clearly indicate that the aerobic and anaerobic cultures are significantly different with respect of their capability to reduce XTT.

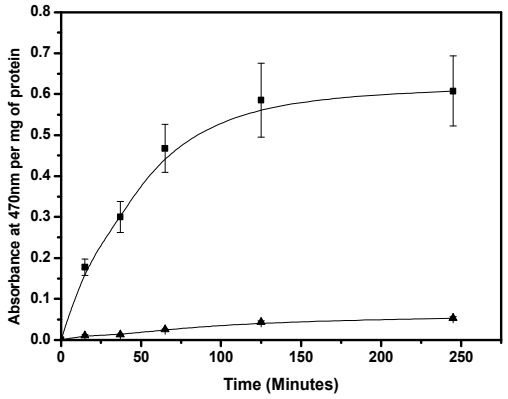
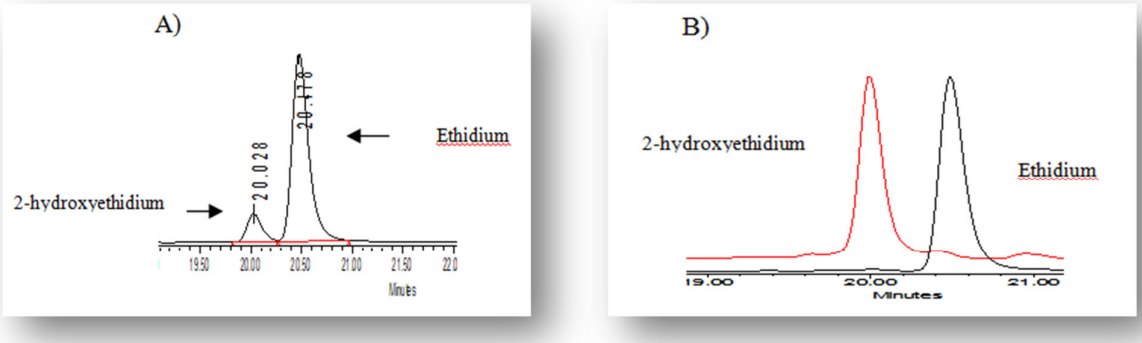


Figure 2.3: Comparison of superoxide production by active and dormant culture of *Mycobacterium smegmatis*. (A) Comparison of XTT reduction by active and dormant culture of *Mycobacterium smegmatis*.XTT at a final concentration of 100µM was added through a syringe into the (■) Wayne model tube on 7th day and directly to a (▲) 1 OD_{620} culture of *M. smegmatis*. Incubation was done at different time points at 37°C and absorbance was taken at 470nm. Superoxide concentration was determined as described in "Materials and Methods". The results are shown as average of three identical experiments with S.D.

2.3.3 Detection of superoxide by active and dormant *Mycobacterium smegmatis* cells using DHE-HPLC

In order to confirm the above findings that superoxide is produced in active and dormant *M. smegmatis* bacilli, we used another dye, dihydroethidium (DHE). DHE, by virtue of its ability to freely permeate cell membranes is used extensively to monitor superoxide production. DHE upon oxidation yields two fluorescent products 2-hydroxyethidium (Oxyethidium), specific for superoxide and the less-specific product ethidium [29]. By using HPLC, the reaction products 2-hydroxyethidium and ethidium, were successfully separated at retention times 20.02 and 20.47 minutes respectively (Figure 2.4).



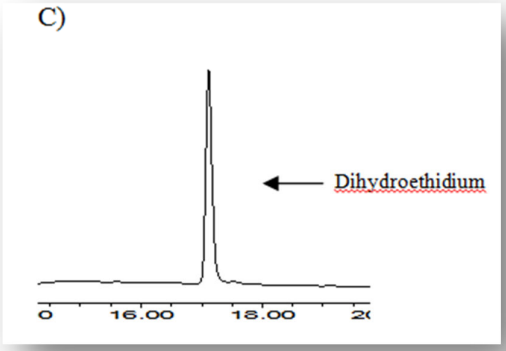


Figure 2.4: Chromatographic separation of 2-hydroxyethidium and ethidium. A) HPLC chromatogram showing separation of 2-hydroxyethidium and ethidium peaks. Identification was performed with fluorescence detector (W2475) at excitation: 480nm and emission: 580nm. B) Overlay of 2-hydroxyethidium and ethidiumpeaks using standard solutions.Both standards wererun separately under the chromatographic conditions described in Materials & Methods.C) HPLC chromatogram showing dihydroethidium peakobtained by UV detection (245 nm) under chromatographic conditions described in the text.

The concentration of Oxyethidium formed in the cells was measured from a standard plot obtained by using different concentrations of Oxyethidium (2-hydroxyethidium) verses peak area (Figure 2.5).

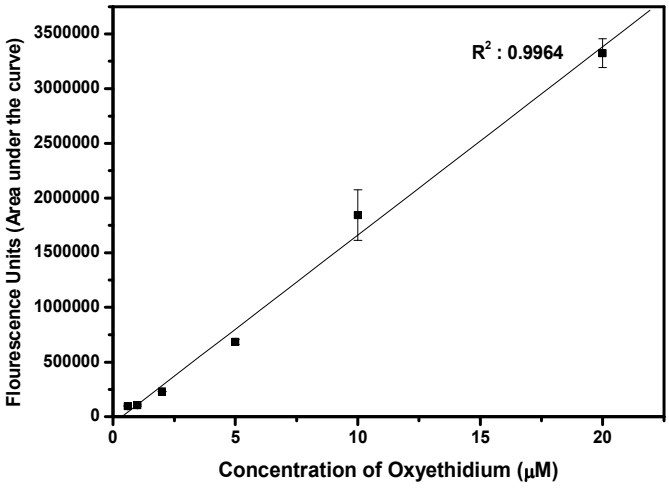


Figure 2.5: Dose response curve of Oxyethidium (standard). Different concentrations of Oxyethidium (EOH) were made in water and run on HPLC system(Waters). The area under the curve of Oxyethidium peak is plotted on Y axis and different concentrations of Oxyethidium are plotted on X axis. The results are shown as average of three identical experiments with S.D.

It was observed that superoxide production was steadily increased with time when different number of *M. smegmatis* cells (0.3, 0.6 and 1.0 OD_{620}) were used (Figure 2.6A). A time dependent increase in Oxyethidium production indicates superoxide production by the aerobically growing *M. smegmatis* cells (Figure 2.6B).

(A) (B)

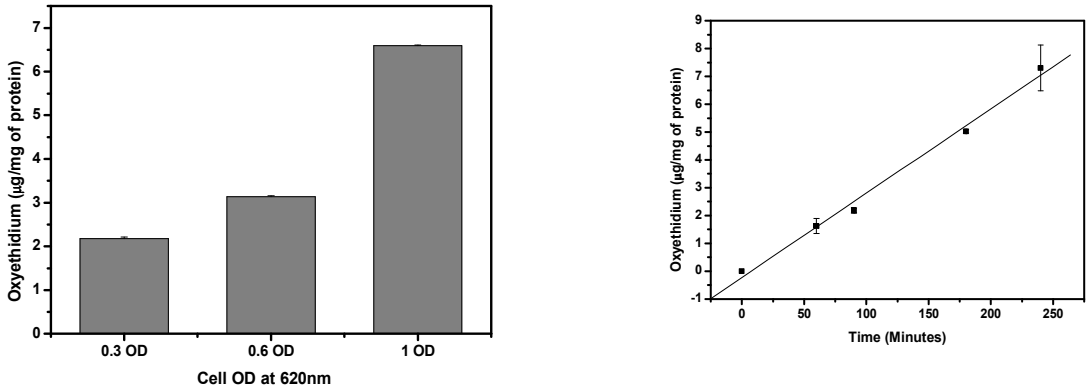


Figure 2.6: Detection of superoxide production by *Mycobacterium smegmatis* cells. A) Effect of different concentrations of cells on superoxide production in terms of Oxyethidium produced by *M. smegmatis*. Log cultures of 0.3 OD_{620} , 0.6 OD_{620} and 1 OD_{620} of *M. smegmatis* were treated with DHE to achieve a concentration of 25µM and the rest of the protocol was followed as described in Materials and Methods. After filtration, the extract was injected into the HPLC system (Waters), as described in text, and identification was performed with fluorescence detector (W2475) at excitation : 480nm and emission : 580nm. (B) Time kinetics of superoxide production in terms of Oxyethidium produced by aerobically growing *M. smegmatis* cells. Log cultures of 0.3 OD_{620} of *M. smegmatis* were treated with DHE to achieve a concentration of 25µM and the rest of the protocol was followed as described in Materials and Methods. After filtration, the extract was injected into the HPLC system (Waters), as described in text, and identification was performed with fluorescence detector (W2475) at excitation : 480nm and emission : 580nm. The results are shown as average of three identical experiments with S.D.

Superoxide production was also detected and measured in dormant *M. smegmatis*. To do this, 7^{th} day old Wayne model culture was used. Superoxide production was found to be more in dormant cells than actively growing bacilli (Figure 2.7).

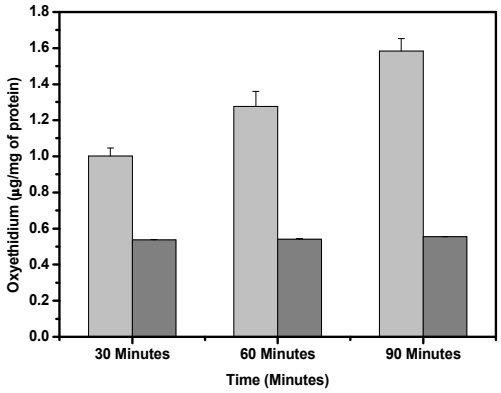


Figure 2.7:Comparison of DHE reduction by active and dormant *Mycobacterium smegmatis* using HPLC. 25µM of DHE was incubated with (Dark gray) 0.3 OD_{620} of aerobically growing *M. smegmatis* cells and (Light gray) 7th day Wayne hypoxia model. Rest of the protocol was followed as described in "Materials and Methods". After filtration, the extract was injected into the HPLC system (Waters), as described in text, and identification was performed with fluorescence detector (W2475) at excitation : 480nm and emission : 580nm. The results are shown as average of three identical experiments with S.D.

Superoxide production was further validated and visualized by fluorescent microscopy. The reaction product, 2-hydroxyethidium, intercalates with DNA and is well retained by the cells. Thus, images of fluorescent cells as shown in figure 2.8 confirm production of superoxide by active and dormant *M. smegmatis*.

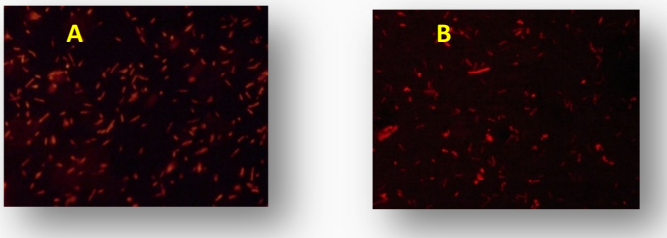


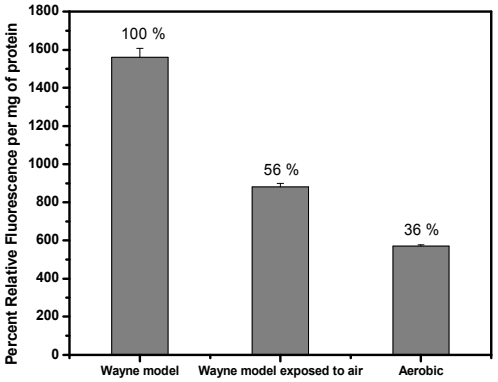
Figure 2.8: Detection of superoxide production by active and dormant cultures of *Mycobacterium smegmatis* using DHE-Microscopy. DHE was added at a final concentration of 10µM to (A) 1 OD_{620} culture of actively growing *M. smegmatis* and (B) 7th day old hypoxic Wayne model culture of *M. smegmatis*. Samples were prepared and images were taken as described in "Materials and Methods".

2.3.4 Effect of aeration on ROS formation in dormant culture of *Mycobacterium smegmatis*

As DCFH-DA oxidation and XTT reduction were found to be higher in dormant culture than aerobic culture, to see whether it is dependent on amount of oxygen, we kept the Wayne model (dormant) culture in aerobic condition for 3hours

and then measured DCFH-DA oxidation and XTT reduction. The amount of DCFH-DA oxidation was drastically reduced by 44% in aerated culture as compared to non aerated culture (Figure 2.9A). The amount of XTT reduced also decreased by 77% in aerated culture compared to non aerated culture (Figure 2.9B) which indicates that this property of DCFH-DA oxidation and XTT reduction is vulnerable to the amount of aeration and hypoxia is required for superoxide generation.

(A)



(B)

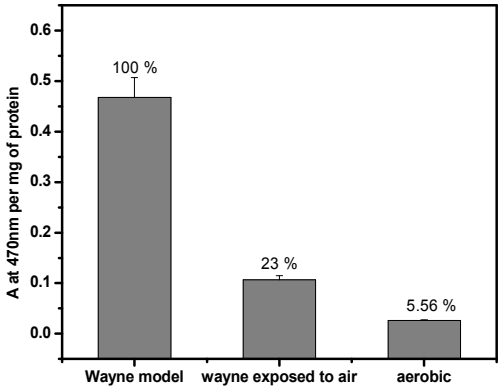


Figure 2.9: Essential role of hypoxia in ROS production by dormant *Mycobacterium smegmatis*. (A) Effect of aeration on DCFH-DA oxidation in dormant culture of M. *smegmatis*. 1 OD_{620} of aerobically growing *M. smegmatis*, 7th day hypoxic culture of Wayne model and 7th day hypoxic culture of Wayne model exposed to air for 3 hours were incubated with 20μM DCFH-DA for 30 minutes at 37°C and fluorescence measurements were taken as described in "Materials and Methods". The percent relative fluorescence per well was calculated by formula [(Ft – Ft_0)/ Ft_0*100], where Ft = fluorescence at time t and Ft_0 = Fluorescence at time 0 min. (B) Effect of aeration on XTT reduction by dormant culture of *M. smegmatis*. 1 OD_{620} of aerobically growing *M. smegmatis*, 7th day culture of Wayne model and 7th day culture of Wayne model exposed to air for 3 hours were incubated with

100µM XTT for 30 minutes at 37°C and absorbance was taken as described in "Materials and Methods". All the experiments were performed in triplicates. Results are mean values with SD.

2.3.5 Effect of ROS modulators on superoxide production by active and dormant *Mycobacterium smegmatis*

In order to further investigate the possible relation between growth inhibitory activity of different ROS modulators (data not shown here) and superoxide level in the culture, the level of free superoxide radicals was estimated by adding DHE in the culture medium of actively growing bacilli treated with various ROS modulators (Table 2.1). ROS modulators were used at their respective IC_{90} values which were determined against aerobically growing *M. smegmatis* bacilli in our laboratory (Data not shown here). Ascorbic acid, p-coumaric acid and caffeic acid, known antioxidants [30-32] reduced the level of free superoxide by 89.2%, 33.1% and 49.1% respectively when applied at their respective IC_{90} values in the culture. Further, we treated *M. smegmatis*culture with menadione (superoxide generator) [33, 34] and found an increase in superoxide levels by 15%. Also, trien (superoxide dismutase inhibitor) [35] increased the production of superoxide by 195.2% which clearly strengthened the hypothesis that superoxide is actively produced by *M. smegmatis* cells. Therefore, our results so far strongly suggests that actively growing *M. smegmatis* bacilli produce significant level of superoxide which is possibly linked to its growth.

Table 2.1: Effect of different ROS modulators on superoxide production by aerobically growing *Mycobacterium smegmatis*

ROS Modulators (mM)[a]	% Inhibition ± S.D.
Ascorbic acid (1.64)	89.2 ±26.5
p-Coumaric acid (1.34)	33.1 ± 3.2
Caffeic acid (2.71)	49.1 ± 10.6
Menadione (0.07)	-15.9 ± 1.0
Trien (1.77)	-195.2 ± 19.5

[a]: Compounds are added at their respective IC_{90} values determined against *M. smegmatis*.

% Inhibition is calculated using the formula [(control-test)/(control-blank)].

Control consisted of cells and vehicle (DMSO). Blank is zero time measurement without any incubation.

The results are shown as average of three identical experiments with S.D.

To further confirm the presence of superoxide and determine its source in dormant culture of *M. smegmatis*, different ROS modulators were used in XTT reduction assay. It was found that Tempol, which is a superoxide mimic [36], inhibits XTT reduction by 97%. DPI which is an NADH oxidase inhibitor [37-39] was found to inhibit XTT reduction by 75%. 2-MP (2-Mercaptopyridine), inhibitor of fumarate reductase [40], was found to increase XTT reduction by 68% whereas p-coumaric acid, an antioxidant was found to inhibit XTT reduction by 22% (Table 2.2).

Table 2.2: Effect of ROS modulators on XTT reduction by dormant culture of *Mycobacterium smegmatis*

ROS Modulators	**% Inhibition[b]**
Control	0.00
DPI (10µg/ml)	75.32
2 MP (250µM)[a]	-68.7
Tempol (9.01mM)[a]	97.1
p-Coumaric acid (1.34mM)[a]	22.2

[a]Compounds are added at their respective IC_{90} values determined against *M. smegmatis*.

[b]% Inhibition is calculated using the formula [(control-test)/(control-blank)].

Control consisted of cells and vehicle (DMSO). Blank is zero time measurement without any incubation.

The results are shown as average of three identical experiments with S.D.

2.3.6 NADH oxidase as the major source of superoxide in aerobically growing *Mycobacterium smegmatis*

Fumarate reductase and terminal quinol oxidase associated with ETC were earlier shown to be major sources of superoxide production in *E. coli* and *E. faecalis*respectively [41, 42]. Although fumarate reductase is an essential enzyme in hypoxia-induced dormant mycobacterium, its functional role as a superoxide

generator is not yet established in mycobacteria [43]. In addition, a functional NADH oxidase gene, which is a bacterial homologue of NADPH oxidase in mammalian cells, was found to be present in *M. smegmatis*[44].

In order to identify the possible source(s) of superoxide in aerobically growing *M. smegmatis* cells, we tested the effect of different substrates and inhibitors on the oxidation of DHE using *M. smegmatis* cells (Figure 2.10). When NADH was added as a substrate to the incubation mix, superoxide production was increased by 9% in presence of NADH (0.1mM) as compared to control cells and it increased by 57% with 1mM NADH. Succinate and fumarate also showed increase in DHE oxidation but it was not as significant as NADH. Additionally, DHE oxidation was inhibited up to 81% by DPI, which is mainly an NADH oxidase inhibitor and 58% by 2MP, which is a fumarate reductase inhibitor (Figure 2.11). ETC inhibitors, such as rotenone (complex I) and antimycinA (complex III) decreased superoxide production by 28% and 17% respectively, indicating that the ETC is a minor contributor to the total pool of superoxide.

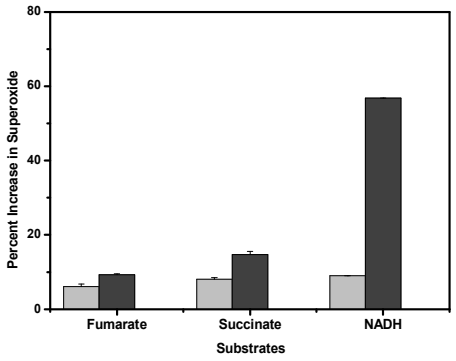


Figure 2.10: Effect of different substrates on superoxide production by aerobically growing *Mycobacterium smegmatis* cells. 0.3 OD_{620} of *M. smegmatis* cells were incubated with (light grey) 0.1mM and (dark grey) 1mM of Fumarate, succinate and NADH respectively. Superoxide estimation was done as described in "materials and methods". % inhibition is calculated using the formula [(control-test)/(control-blank)]. Control consisted of cells, vehicle (DMSO) and DHE whereas blank consisted of vehicle and DHE only. The results are shown as average of three identical experiments with S.D.

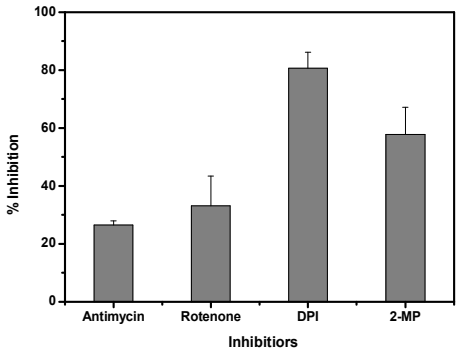


Figure 2.11: Effect of different inhibitors on superoxide production by aerobically growing *Mycobacterium smegmatis* cells. 0.3 OD_{620} of aerobically growing *M. smegmatis* cells were treated separately with antimycinA A (20µM), rotenone (200µM), DPI (10µg/ml) and 2-MP (250µM) for 5 minutes and then DHE at a final concentration of 25µM were added and further incubation was done for 3 hours at 37°C. % inhibition is calculated using the formula [(control-test)/(control-blank)]. Control consisted of cells, vehicle (DMSO) and DHE whereas blank consisted of vehicle and DHE only. The results are shown as average of three identical experiments with S.D.

2.3.7 Superoxide production from membrane bound NADH oxidase in aerobically growing *Mycobacterium smegmatis*

It is already noticed that NADH has possibly the major role in superoxide production within *M. smegmatis*. As NADH oxidase is a membrane bound protein [45], superoxide production is likely to take place by the membrane. We prepared *M. smegmatis* crude membrane by applying sphaeroplast solution as described in "Materials and Methods". Before going for superoxide detection by HPLC method which is a tedious procedure, we determined NADH utilization by *M. smegmatis* crude membrane. The crude membrane was able to utilize NADH in a time dependent manner (Figure 2.12) which ensures that the NADH utilizing enzymes are active in the membrane.

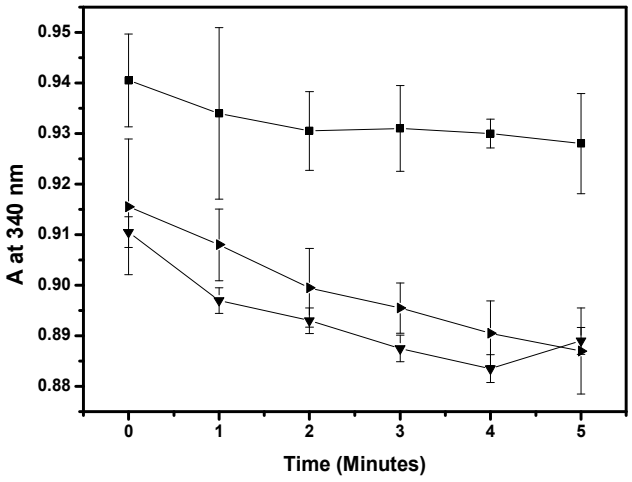


Figure 2.12: NADH utilization by *Mycobacterium smegmatis* crude membrane. 20µl of (■) 0.4µg/ml of membrane protein, (▽) 0.8µg/ml of membrane protein and 1.6µg/ml of membrane protein were incubated with 210µM NADH in *M. phlei* medium and absorbance was monitored at 340nm for 5 minutes period in a kinetic mode. The results are shown as average of three identical experiments with S.D.

As DPI is a specific inhibitor of NADH oxidase and it has been shown to decrease superoxide production by whole cells of *M. smegmatis*, we used DPI to see its effect on NADH utilization by crude membrane preparation. DPI was able to inhibit NADH utilization in a concentration dependent manner. This indicates that the NADH utilization by membrane is mostly occurring because of NADH oxidase (Figure 2.13).

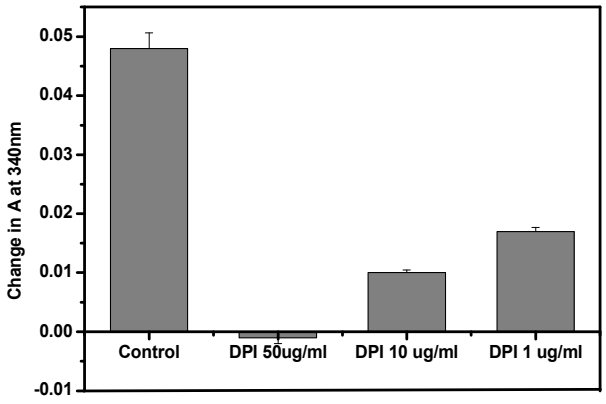


Figure 2.13: NADH utilization by *Mycobacterium smegmatis* crude membrane preparation in presence of DPI.20µl of 0.8µg/ml of membrane protein was incubated with 210µM NADH in *M. phlei* medium in presence of different concentrations of DPI and absorbance was monitored at 340nm for 5 minutes period in a kinetic mode. Change in absorbance was calculated using the

formula (T0-T5), where T0 is the absorbance at 0 minute and T5 is the absorbance at 5 minutes. The results are shown as average of three identical experiments with S.D.

Then we determined superoxide production in terms of Oxyethidium produced by *M. smegmatis* crude membrane preparation. It was observed that superoxide was produced by crude membrane preparation in a time dependent and membrane concentration dependent manner when membrane preparation along with NADH as substrate was incubated with DHE (Figure 2.14). Additionally, this superoxide production was substantially inhibited by DPI which is corroborating our earlier observation of NADH oxidase as major contributor of superoxide pool in *M. smegmatis* culture (Figure 2.15)

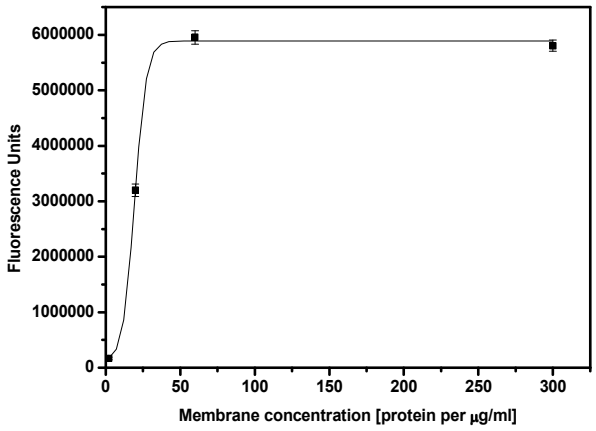


Figure 2.14: DHE reduction by *Mycobacterium smegmatis* crude membrane preparation. DHE at a final concentration of 25µM was incubated with different concentrations of *M. smegmatis* crude membrane preparations in presence of 210µM NADH. After 90 minutes of incubation samples were prepared for HPLC as described in "Materials and Methods". 0 minute reading without any incubation served as blank. The results are shown as average of three identical experiments with S.D.

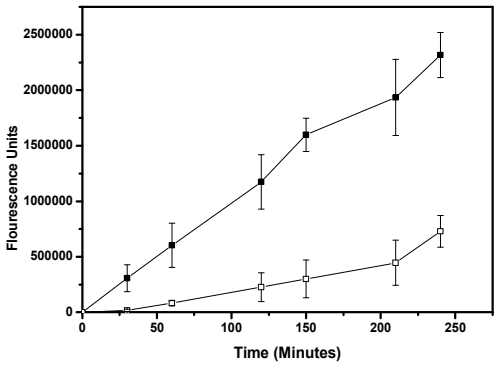


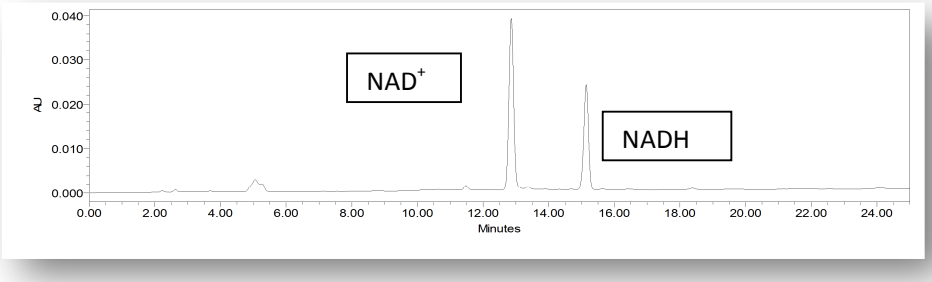
Figure 2.15: Kinetics of superoxide production by *Mycobacterium smegmatis* crude membrane preparation.10µg/ml of DPI is incubated with *M. smegmatis* membrane (20 µl of 0.8µg/ml of protein) preparation (□) for 5 min. In control, the membrane is

incubated with DMSO only (■). DHE at a final concentration of 25µM was added to this reaction mixture and HPLC chromatograms samples were prepared as described in "Materials & Methods". The results are shown as average of three identical experiments with S.D.

2.3.8 Estimation of NAD^+/NADH ratio in active and dormant *M. smegmatis* cells

The NAD^+/NADH ratio in active and dormant stages could possibly provide a useful indication of the metabolic activity as well as justification behind the burst of superoxide release by the *M.smegmatis*culture under anaerobic condition. NAD^+ absorbance is monitored at 260 nm and the retention time obtained was 12.57 minutes whereas NADH absorbance was monitored at 340 nm and the retention time was obtained at 15.2 minutes (Figure 2.16). NAD^+ and NADH values were calculated from the standard graph (Figure 2.17).

(A)



(B)

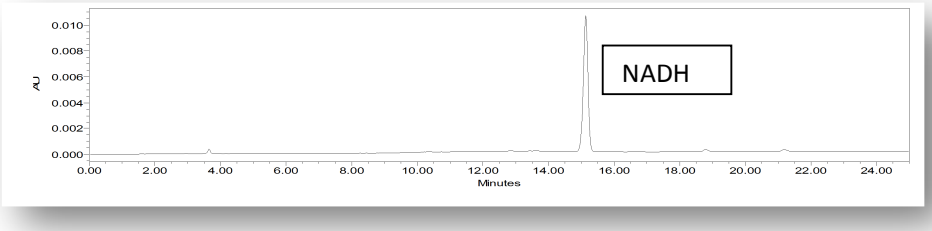


Figure 2.16: Chromatographic separation of NAD^+ and NADH. A) HPLC chromatogram showing NAD^+ peak at 260nm. B) HPLC chromatogram showing NADHpeak at 340nm.

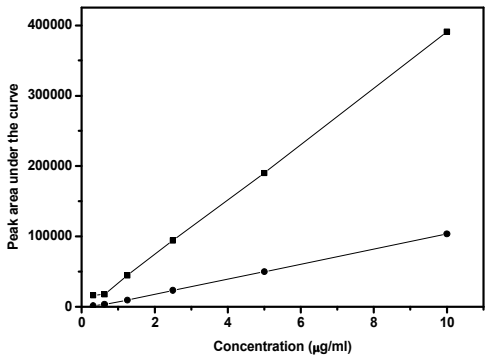


Figure 2.17: Dose response curve of NAD^+ and NADH (standard). Different concentration of NAD^+ (■) and NADH (●) were made in water and run on HPLC system (Waters). The area under the curve is plotted on Y axis and different concentrations of NAD^+ and NADH are plotted on X axis.

$NADH/NAD^+$ ratios were calculated for active as well as dormant culture. The ratio value was found to be higher in dormant culture than in active culture (Figure 2.18). This corroborates with the earlier observations that in dormancy more reducing equivalents are generated and NADH could act as a possible source of superoxide under anaerobic conditions [45, 46].

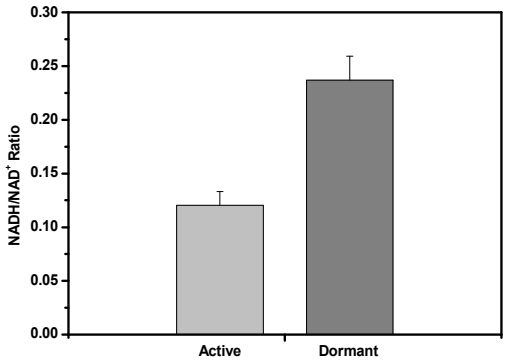


Figure 2.18: Comparison of $NADH/NAD^+$ ratio in active and dormant *M. smegmatis* culture.

O.46 OD_{620} of aerobically growing *M. smegmatis* cells (Light gray) and 0.46 OD_{620} of Wayne model (7^{th} day) cells (Dark gray) were taken to extract NAD^+ and NADH as described in Materials and Methods. Chromatographic separation and peak area determination of NAD^+ and NADH was done and therespective concentrations were found out using standard curves given in Figure 2.17. NAD^+ absorbance was monitored at 260 nm whereas NADH absorbance was monitored at 340 nm.The results are shown as average of three identical experiments with S.D.

2.4 Discussion

Recent studies have highlighted the significant role of ROS as physiological regulators of intracellular signalling pathways [46]. ROS mediated signalling is controlled by a delicate balance between its formation and scavenging [47]. In this study, we endeavoured to find out if *M. smegmatis* produces superoxide and its possible sites of production. We have also compared total ROS levels followed by specific emphasis on superoxide production in active and dormant *M. smegmatis* cultures.

Our initial studies using DCFH-DA has clearly indicated that actively growing *M. smegmatis* produces significant amount of ROS (Figure 2.1). As ROS comprises of superoxide, hydrogen peroxide and hydroxyl radicals, it is important to determine the presence and levels of these molecules individually. Superoxide is the first radical to form from molecular oxygen, which gets converted to hydrogen peroxide by SOD and then to hydroxyl radical by fenton reaction. Thus, being the first radical to form, its study becomes very important. We determined superoxide levels by using XTT and a very specific fluorescent probe for superoxide, DHE. All these studies have clearly established that actively growing *M. smegmatis* bacilli continuously produce superoxide radicals to a significant level which are inhibited by ROS modulators (Figures 2.3, 2.6, 2.8 and Table 2.1&2.2).Superoxide production is found to occur at a linear rate from whole cells as well as membrane fragments indicating membrane as the site of its production in *M. smegmatis*(Figures 2.6, 2.14&2.15). Earlier studies have shown that significantly less production of ROS in the D9 macrophage cell line and mice deficient in the p47 or gp91 subunits of NADPH oxidase result in resistance to Mtb infection [48-51]. It is also shown that antioxidants have both *in vitro* as well as *in vivo* anti-mycobacterial activity [52, 53]. *In vivo* studies have reported that antioxidants [MnTE-2-PyP (manganese (II) meso-tetrakis-(N-Methypyridinium -2-*yl*) porphyrin) and N-acetyl-l-cysteine] inhibit the growth of *M. abscessus* in macrophages [54]. Our finding indicates that antioxidants scavenge superoxide and prompt us to hypothesize that the inhibitory activity could be because of scavenging superoxide (Table 2.1).

It was earlier reported that the amount of superoxide formed is proportional to the amount of oxygen available to the cells [55-57]. Therefore, one can hypothesize that superoxide levels would decrease significantly as hypoxia is achieved. However,

we observed more ROS and superoxide in Wayne's dormant culture than in aerobically growing culture.

Induction of ROS in hypoxia needs to be studied in detail. Numerous observations are now coming up showing ROS burst during hypoxia. In a study on skeletal muscles, it was shown that they behave similar to heart muscles. In the beginning of hypoxia a transient ROS burst was observed while continuous production of ROS was seen in chronic exposure to very severe hypoxia [57]. Mitochondria were shown to produce transient ROS after exposure to hypoxia [56]. Cellular hypoxia is usually described as a form of "reductive stress". When insufficient O_2 is available for reduction by the electron transport chain, more reducing equivalents begin to accumulate (Figure 2.19). Thus, more electrons are made available for reduction reactions like reduction of O_2 to superoxide due to accumulation of reducing equivalents [57]. Therefore, we also measured NADH/NAD^+ ratio in aerobically growing and Wayne Model dormant bacilli, and it was found that the ratio is higher in Wayne Model dormant culture than in aerobic culture (Figure 2.18) which corroborates with the reported observations [58, 59].

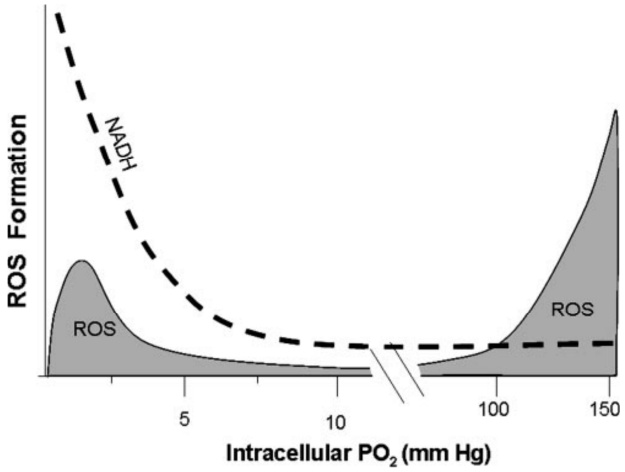


Figure 2.19: Depiction of the proposed bimodal distribution of reactive oxygen species (ROS) formation. Extracted from Clanton T.L., J. Apply. Physiol., 102:2379-2388, 2007.

Wayne et al have developed a model to study latency in mycobacteria in which, a gradual depletion of oxygen leads to dormancy phenotype. To compare level of superoxide in active and dormant *M. smegmatis*, we used Wayne's dormancy model and nutrient starvation model to compare with actively growing bacilli. We observed more ROS and more superoxide in case of Wayne's model compared to actively growing bacilli and nutrient starved bacilli (Figure 2.1, 2.2, 2.3,

2.7&2.8).Interestingly the superoxide formation is suppressed in dormant cultures (Wayne Model) which were exposed to air (Figure 2.9). This led us to speculate that the burst of ROS is strongly dependent on hypoxia. There are two possibilities. One, there is sufficient O_2 and NADH available in Wayne's dormant culture to form superoxide or second could be that the extraneous addition of reagents is adding little oxygen to the anaerobic stage dormant bacilli and the transient hypoxia thereby produced is responsible for the apparent ROS burst which we have seen. In Wayne dormant culture we have seen accumulation of more NADH compared to aerobically growing bacilli. Thus, the added oxygen can be utilized by NADH oxidase to give a burst of superoxide. In this case, it is important to measure the oxygen concentrations in Wayne dormancy model before and after addition of reagents. If it is the second case, then this hypoxic burst of ROS could play important role in resuscitation of these dormant bacilli. This will be clear with further studies.

A suitable candidate capable of generating superoxide would be one similar to eukaryotic NADPH oxidases, known to be prolific superoxide generators [60]. A significant increase in superoxide production is found to occur in presence of NADH (57%), succinate (15%) and fumarate (9%) indicating NADH dependent oxidase/dehydrogenase as the major site of superoxide production (Figure 2.10). NADH dehydrogenase and NADH oxidase is specifically inhibited by rotenone and DPI respectively [61-63]. Our results clearly indicated that DPI exhibits profound inhibitory effect on superoxide production by whole cells (81%) as well as membrane fragments in presence of NADH (Figure 2.11, 2.13, 2.15). Thus, NADH oxidase could be the primary source of superoxide production in *M. smegmatis*. In a study published by Julia M. Diaz et al, out of 30 heterotrophic bacteria studied, 27 were reported to produce extracellular superoxide and NADH oxidase was identified as the source of this superoxide in a model organism *Roseobacter clade* [64]. Interestingly, *M. smegmatis* possess four isozymes of NADH oxidase as seen in the KEGG database. Further studies on these NADH oxidases will clarify which of these is responsible for superoxide production. Other sources of superoxide include leakage of electrons from components of the ETC such as NADH dehydrogenase, succinate dehydrogenase (which also functions as fumarate reductase in *M. smegmatis)* [65] and cytochrome bc1 complex [66]. But lack of any significant effect by ETC inhibitors like rotenone and antimycinA on superoxide production clearly indicated that they

might be minor contributors of superoxide produced in the cell under the conditions applied (Figure 2.10). However, 2MP, a fumarate reductase inhibitor was found to inhibit superoxide production by 58% and hence acts as a secondary contributor (Figure 2.11). To find out the source of superoxide in Wayne model, XTT assay was performed with different ROS modulators. The data (Table 2.2) need further confirmation by using DHE HPLC method; however, inhibition of XTT reduction by 97% by Tempol (SOD mimic) confirms that the XTT reduction is mainly due to superoxide. In Wayne model of dormancy, we observed inhibition of superoxide mainly by DPI (75%) which corroborates with our observations in aerobic cultures but, interestingly we found more superoxide formation after addition of 2MP, fumarate reductase inhibitor. This is interesting because, in aerobic condition 2MP is inhibiting superoxide production whereas in Wayne *in vitro* model, it is enhancing superoxide formation (Figure 2.11 & Table 2.2). Fumarate could act as a final electron acceptor in ETC under anaerobic condition [67, 68]. So, fumarate will accept electron and get converted to succinate by fumarate reductase enzyme. If this enzyme is blocked by 2MP, then the final step will be blocked. And thus, all the reducing equivalents (mainly NADH) going into the ETC will get diverted to NADH oxidase and in turn produce superoxide. This is how we might be getting more superoxide production after treatment with 2MP in dormant culture. If this hypothesis is true, then one should get more superoxide by using any inhibitor which inhibits the final step in ETC in hypoxic condition e.g. Nitrate reductase inhibitor because nitrate can also work as a final electron acceptor in anaerobic conditions [15]. This can be tested. More studies on role of fumarate reductase in active and dormant stage with respect to superoxide production could lead to some interesting result that will help in understanding the transition of mycobacteria from active to dormant stage and vice-a-versa.

The significant production of superoxide by *M. smegmatis*also justifies the presence of an array of antioxidant enzymes reported to be expressed by mycobacteria. Antioxidant enzymes, such as SodC, KatG, and AhpC in mycobacteria are also known to play an important role in defence against ROS stress in the host [69]. But, in light of current finding, these antioxidant enzymes could be essential in maintaining the pathogens own redox balance. The antioxidant enzyme gene cluster was transiently up-regulated when tubercle bacteria stopped growing in mouse lung,

which further shows the essential role of ROS during active growth under aerobic conditions [69]. In this context, it should also be noted that vitamin D, a potent antioxidant, was earlier used for the treatment of tuberculosis, and its deficiency has recently been identified as a major cause of prevalence of the disease in South African population [70]. It has been generally observed that patients suffering from tuberculosis have high oxidative stress and lower antioxidant levels in blood serum [71]. The target and actual role of these antioxidants may now be envisaged in context of current findings.

In summary, this study clearly shows that a significant amount of superoxide is actively produced by *M. smegmatis*; mainly by NADH oxidase of the bacilli. The comparative study between active and dormant *M. smegmatis* bacilli shows that the dormant (Wayne Model) bacilli apparently produce significantly higher amount of superoxide than the actively growing bacilli. This need further studies to find the reasons and its implications in resuscitation of dormant bacilli.

2.5 References

1) Ehrt S. and Schnappinger D.,Mycobacterial survival strategies in the phagosome: defence against host stresses,Cell.Microbiol., 11:1170–1178, 2009.
2) Edwards K.M., Cynamon M.H., Voladri R.K.R., Hager C.C., DeStefano M.S., Tham K.T., Lakey D.L., Bochan M.R. and Kernodle D.S., Iron-cofactored superoxide dismutase inhibits host responses to *Mycobacterium tuberculosis*,Am j. Respir. Crit., Care Med., 164:2213–2219, 2001.
3) Piddington D.L., Fang F.C., Laessig T., Cooper A.M., Orme I.M. and Buchmeier N.A., Cu,Zn Superoxide dismutase of *Mycobacterium tuberculosis* contributes to survival in activated macrophages that are generating an oxidative burst, Infect.and Immun., 69:4980–4987, 2001.
4) Dussurget O., Stewart G., Neyrolles O., Pescher P., Young D. and Marchal G., Role of *Mycobacterium tuberculosis* copper–zinc superoxide dismutase, Infect. and Immun., 69:529–533, 2001.
5) Fang F.C., Antimicrobial reactive oxygen and nitrogen species: concepts and controversies,Nat. Rev. Microbiol., 2:820-832, 2004.
6) Ng V.H., CoxJ.S., Sousa A.O., MacMickingJ.D. and McKinneyJ.D., Role of KatG catalase-peroxidase in mycobacterial pathogenesis: countering the phagocyte oxidative burst,Mol.Microbiol., 52:1291–1302, 2004.
7) Bartos M., FalkinhamJ.O. and Pavlik I., Mycobacterial catalases, peroxidases, and superoxide dismutases and their effects on virulence and isoniazid-susceptibility in mycobacteria – a review,Vet. Med. (Czech), 49:161–170, 2004.
8) Hugo M., Radi R. and Trujillo M., Thiol-dependent peroxidases in *Mycobacterium tuberculosis* antioxidant defense, Understanding Tuberculosis-Deciphering the secret life of the bacilli, Dr. Pere-Joan Cardona (Ed.), ISBN: 978-953-307-946-2, InTech, Available from: http://www.intechopen.com/books/understanding-tuberculosis-deciphering-the-secret-life-of-the-bacilli/thiol-dependent-peroxidases-in-mycobacterium-tuberculosis-antioxidant-defence.
9) Sareen D., Newton G.L., Fahey R.C. and Buchmeier N.A., Mycothiol is essential for growth of *Mycobacterium tuberculosisErdman*,j. of Bacteriol., 185:6736–6740, 2003.
10) Rawat M., Newton G.L., Ko M.,Martinez G.J., Fahey R.C. and Av-Gay Y., Mycothiol-Deficient *Mycobacterium smegmatis* mutants are hypersensitive to

alkylating agents, Free Radicals and Antibiotics,Antimicrob. Agents and Chemotherapy, 46:3348–3355, 2002.

11) Autréaux B.D. and Toledano M.B., ROS as signaling molecules: mechanisms that generate specificity in ROS homeostasis,Nat. Rev.: Mol. Cell. Biol., 8:813-824, 2007.

12) Trachootham D., Lu W., Ogasawara M.A., Valle N.R. and Huang P., Redox regulation of cell survival,Antioxid. redox signalling, 10:1343-1374, 2007.

13) Turrens J.F., Mitochondrial formation of reactive oxygen species, j. Physiol., 552:335-344, 2003.

14) Nicolopoulou-Stamati N., Hens L., Howard V.C. and Larebeke N.V., Cancer as an environmental disease, Springer, 20:93, 2004.

15) Khan A. and Sarkar D., Identification of a respiratory-type nitrate reductase and its role for survival of *Mycobacterium smegmatis*in Wayne model, Microb. Pathog., 41:90-95, 2006.

16) Bagchi G.B., Das T.K. and Tyagi J.S., Molecular analysis of the dormancy response in *Mycobacterium smegmatis*: expression analysis of genes encoding the DevR-DevS two component system, Rv3134c and chaperone K-crystallin homologues, FEMS Microbiol. Lett., 211:231-237, 2002.

17) Dick T., Lee B.H. and Murugasu O.B., Oxygen depletion induced dormancy in *Mycobacterium smegmatis*, FEMS Micro. Biol. Lett.,163:159-164, 1998.

18) Anuchin A. M., Mulyukin A.L., Suzina N.E., Duda V.I., Ei-Registan G.I. and Kaprelyants A.S., Dormant forms of *Mycobacterium smegmatis*with distinct morphology, Microbiol., 155:1071-1079, 2009.

19) Wayne L.G. and Hayes L.G., Nitrate reduction as a marker for hypoxic shift down of *Mycobacterium tuberculosis*, *Tubercle Lung Dis*.,79**:**127-32, 1998.

20) Betts J.C., Lukey P.T., Robb L.C., McAdam R.A. and Duncan K., Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling, *Mol. Microbiol,* 43:717-731, 2002.

21) Wayne L.G. and Hayes L.G., An in vitro model for sequential study of shift down of *Mycobacterium tuberculosis* through two stages of non-replicating persistence, *Infect. Immun,* 64**:** 2062-2069, 1996.

22) Brennan R.J. and Schiestl R.H., Aniline and its metabolites generate free radicals in yeast, Mutagenesis, 12:215-220, 1997.

23) Able A.J., Guest D.I. and Sutherland M.W., Use of a New Tetrazolium-Based Assay to Study theProduction of Superoxide Radicals by Tobacco Cell Cultures Challenged

with Avirulent Zoospores of Phytophthoraparasiticavarnicotianae, Plant Physiol., 117:491-499, 1998.

24) Zhao H., Kalivendi S., Zhang H.,JosephJ., Nithipatikom K., V´Asquez-VivarJ. and Kalyanaraman B., Superoxide reacts with hydroethidine but forms a fluorescent product that is distinctly different from ethidium: potential implications in intracellular fluorescence detection of superoxide,Free Radic. Biol. and Med., 34:1359–1368, 2003.

25) Laurindo F.R.M., Fernandes D.C. and Santos C.X.C., Assessment of Superoxide Productionand NADPH Oxidase Activity by HPLC Analysis of DihydroethidiumOxidation Products, Methods in Enzymology, 441:238-260, 2008.

26) Akhtar S., Sarkar S., Mishra A. and Sarkar D., A method to extract intact and pure RNAfrom mycobacteria, Anal Biochem., 417:286-8, 2011.

27) Sporty J.L., Kabir M.M., Turteltaub K.W., Ognibene T., Lin S.J. and bench G., Single sample extraction protocol for the quantification of NAD+ and NADH redox states in *Saccharomycescerevisiae,* J Sep Sci., 31:3202-3211, 2008.

28) Singh U., Akhtar S., Mishra A. and Sarkar, D, A novel screening method based on menadione mediated rapid reduction of tetrazolium salt for testing of anti-mycobacterial agents,Microbiol. Methods, 84:202-207, 2011**.**

29) Fernandes D.C., Wosniak Jr. J., Pescatore L.A., Bertoline M.A., Liberman M., Laurindo F.R.M. and Santos C.X.C., Analysis of DHE-derived oxidation products by HPLC in the assessment of superoxide production and NADPH oxidase activity in vascular systems, Am j. Physiol. Cell. Physiol., 292:c413-c422, 2007.

30) Bodannes R.S. and Chan P.C., Ascorbic acid as a scavenger of singlet oxygen, FEBS lett., 105:195-196, 1979.

31) Zang L., Cosma G., GardnerH., Shi H., Castranova V. andVallyathan V., Effect of antioxidant protection by p-coumaric acid on low-density lipoprotein cholesterol oxidation,Am. j. Physiol. Cell. Physiol., 279:C954–C960, 2000.

32) Gülçin I., Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid),Toxicol., 217:213–220, 2006.

33) Cuéllar-CruzM., Castaño I., Arroyo-Helguera O. and De Las Peñas A., Oxidative stress response to menadione and cumene hydroperoxide in the opportunistic fungal pathogen *Candida glabrata*,Mem Inst Oswaldo Cruz, Rio de Janeiro, 104:649-654, 2009.

34) Vattanaviboon P., Panmanee W. and Mongkolsuk S.,Induction of peroxide and superoxide protective enzymes and physiological cross-protection against peroxide killing by a superoxide generator in *Vibrio harveyi*, FEMS Microbiol. Letters, 221:89-95, 2003.

35) De Man J.G., De Winter B.Y., Boeckxstaens G.E., Herman A.G. and Pelckmans P.A., Effect of thiol modulators and Cu/Zn superoxide dismutase inhibition on nitrergic relaxations in the rat gastric fundus, British J. of Pharmacology, 119:1022-1028, 1996.

36) Wilcox C.S. and Pearlman A., Chemistry and Antihypertensive Effects of Tempol and Other Nitroxides, Pharmacol Rev., 60:418-469, 2008.

37) Zhang Y., Wade M.M., Scorpio A., Zhang H. and Sun Z., Mode of action of Pyrazinamide: disruption of *Mycobacterium tuberculosis* membrane transport and energetic by pyrazinoic acid., J. of Antimicrob., Chemotherapy, 52:790-795, 2003.

38) Li N., Yi F., Spurrier J.L., Bobrowitz C.A. and Zou A., Production of superoxide through NADH oxidase in thick ascending limb of Henle's loop in rat kidney, Am. J. Physiol. renal physiol., 282:f1111-f1119, 2002.

39) Ellis J.A., Mayer S.J. and Jones O.T.G., The effect of NADPH oxidase inhibitor diphenelyleneiodonium on aerobic and anaerobic microbicidal activities of human neutrophils, Biochem. j., 251:887-891, 1988.

40) Turrens J.F., Newton C.L., Zhong L., Hernandez F.R., Whitfield J. and Docampo R., Mercaptopyridine-N-oxide, an NADH-fumarate reductase inhibitor, blocks Trypanosomacruzi growth in culture and in infected myoblasts, FEMS Microbiology Letters, 175:217-221, 1999.

41) Imlay J.A., A metabolic enzyme that rapidly produces superoxide, fumarate reductase of *Escherichia coli*, j. Biol. Chem., 270:19767-19777, 1995.

42) Huycke M.M., Moore D., Joyce W., Wise P., Shepard L., Kotake Y. and Gilmore M.S. Extracellular superoxide production by *Enterococcus faecalis* requires demethylmenaquinone and is attenuated by functional terminal quinol oxidases, Mol. Microbiol., 42:729-740, 2001.

43) Watabe M., Zimmermann M., Goodwin M.B., Sauer. U. and Barry C.E., Fumarate reductase activity maintains an energized membrane in anaerobic *Mycobacterium tuberculosis,* PLoSPathog., 7:10, 2011.

44) Rezwan M., Laneelle M.A., Sander P. and Daffe M., Breaking down the wall: fractionation of Mycobacteria, j. Microbiol. Methods, 68:32-39, 2007.

45) Gibbons H.S., Wolschendorf F., Abshire M., Niederweis M. and Braunstein M., Identification of two *Mycobacterium smegmatis* lipoproteins exported by a SecA2-dependent pathway, j. of Bacteriol., 189:5090-5100, 2007.

46) Finkel T., Signal transduction by reactive oxygen species, j. Cell. Biol., 194:7-15, 2011.

47) Bailey-Serres J. andMittler R.,The roles of reactive oxygen species in plant cells,Plant Physiol., 141:311, 2006.

48) ChanJ., Xing Y., Magliozzo R. and Bloom B.R., Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages,j. Exp. Med., 175:1111-1122, 1992.

49) Adams L.B., Dinauer M.C., Morgenstern D.E. and KrahenbuhlJ.L., Comparison of the roles of reactive oxygen and nitrogen intermediates in the host response to *Mycobacterium tuberculosis* using transgenic mice, Tuberc. Lung Dis., 78:237–246, 1997.

50) Cooper A.M., Segal B.H., Frank A.A., Holland S.M., Orme I.M.,Transient loss of resistance to pulmonary tuberculosis in p47phox-/- mice,Infect. and Immun., 68:1231–1234, 2000.

51) Jung Y.J., LaCourse R., Ryan L. and North R.J., Virulent but not avirulent *Mycobacterium tuberculosis* can evade the growth inhibitory action of a T helper 1-dependent, nitric oxide synthase 2-independent defense in mice,j. Exp. Med., 196:991–998, 2002.

52) Hasan N., Yusuf N., Toossi Z. and Islam N., Suppression of *Mycobacterium tuberculosis* induced reactive oxygen species (ROS) and TNF-α mRNA expression in human monocytes by allicin, FEBS Lett., 580:2517–2522, 2006.

53) Reddy M.K., Gupta S.K., Jacob M.R., Khan S.I. and Ferreira D., Antioxidant, Antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punicagranatum,* L. Planta Medica, 73:461-467, 2007.

54) Oberley-Deegan R.E., Rebits B.W., Weaver M.R., Tollefson A.K., Bai X., McGibney M.,Ovrutsky A.R., Chan E.D. and Crapo J.D., An oxidative environment promotes growth of *Mycobacterium abscessus,* FreeRadic. Biol. and Med., 49:1666–1673, 2010.

55) Boveris A., Mitochondrial production of superoxide radical and hydrogen peroxide. In: *Tissue Hypoxia and Ischemia*, edited by Reivich M, Coburn R, Lahiri S, and Chance B. New York: Plenum, p. 67–82, 1977.

56) Korge P., Ping P. and Weiss J.N., Reactive Oxygen Species Production in Energized Cardiac Mitochondria During Hypoxia/Reoxygenation Modulation by Nitric Oxide, Circ. Res., 103:873-880, 2008.

57) Clanton T.L., Hypoxia-induced reactive oxygen species formation in skeletal muscle, *J ApplPhysiol,* 102:2379-2388, 2007.

58) Kumar A., Farhana A., Guidry L, Saini V., Hondalus M. and Steyn A.J.C., Redox homeostasis in mycobacteria:the key to tuberculosis control?, Expert reviews in molecular medicine, 13:e39, 2011.

59) Leonardo M.R., Dailly Y. and Clark D.P., Role of NAD in Regulating the adhE Gene of Escherichia coli, Journal Of Bacteriology, 178:6013–6018, 1996.

60) Bloomfield G. and Pears C., Superoxide signalling required for multicellular development of *Dictyostelium*, j. of Cell. Sci., 116:3387-3397, 2003.

61) Zhang Y., Wade M.M., Scorpio A., Zhang H. and Sun. Z., Mode of action of Pyrazinamide: disruption of *Mycobacterium tuberculosis* membrane transport and energetic by pyrazinoicacid., j. of Antimicrob. Chemotherapy, 52:790-795, 2003.

62) Li N., Yi F., Spurrier J.L., Bobrowitz C.A. and Zou A., Production of superoxide through NADH oxidase in thick ascending limb of Henle's loop in rat kidney, Am. J. Physiol. renal physiol., 282:f1111-f1119, 2002.

63) Ellis J.A., Mayer S.J. and Jones O.T.G., The effect of NADPH oxidase inhibitor diphenelyleneiodonium on aerobic and anaerobic microbicidal activities of human neutrophils, Biochem. j., 251:887-891, 1988.

64) Diaz J.M., Hansel C.M., Voelker B.M., Mendes C.M., Andeer P.F. and Zhang T., Widespread production of extracellular superoxide by heterotrophic bacteria, Sci., 340:1223-1226, 2013.

65) Berney M. and Cook G.M., Unique flexibility in energy metabolism allows mycobacteria to combat starvation and hypoxia, PLoS ONE, 5:e8614, 2009.

66) Chandel N.S., McClintock D.S., Feliciano C.E., Wood T.M., Melendez J.A., Rodriguez A.M. and Schumacker P.T., Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor- 1α during hypoxia, The j. of Biol. Chem., 275:25130-25138, 2000.

67) Guest J.R., Anaerobic Growth of *Escherichia coli* K12 with Fumarate asTerminal Electron Acceptor. Genetic Studies with Menaquinone andFluoroacetate-resistant Mutants, Journal of General Microbiology,115:259-271,1979.

68) Coustou V., Biran M., Besteiro S., Riviere L., Baltz T., Franconi J.M. and Bringaud F., Fumarate Is an Essential Intermediary Metabolite Produced by the Procyclic*Trypanosomabrucei*, J. Biol. Chem., 281:26832-26846, 2006.

69) Shi L., Sohaskey C.D., North R.J. and Gennaro M.L., Transcriptional characterization of the antioxidant response of *Mycobacterium tuberculosis in vivo* and during adaptation to hypoxia *in vitro,* Tuberc., 88:1-6, 2008.

70) Liu P.T., Stenger S., Li H., Wenzel L., Tan B.H., Krutzik S.R., Ochoa M.T., Schauber J., Wu K., Meinken C., Kamen D.L., Wagner M., Bals R., Steinmeyer A., Zügel U., Gallo R.L., Eisenberg D., Hewison M., Hollis B.W., Adams J.S., Bloom B.R. and Modlin R.L., Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response, Sci. 311:1770-1773, 2006.

71) Suresh R. D., Annam V., Pratibha K. and Hamsaveena, Immunological correlation of oxidative stress markers in tuberculosis patients, Int. j. Biol. Med. Res., 1:185-187, 2010.

CHAPTER 3

Induction of Dormancy Phenotype by Inhibiting Superoxide Generation in *Mycobacterium smegmatis*

3.1 Introduction

Mycobacterium tuberculosis (Mtb) is a successful pathogen that causes Tuberculosis(TB). The reason lies in the ability of Mtb to persist within humans for long periods without causing any evident disease symptoms [1]. These dormant non-replicating mycobacteria can revert back to active tuberculosis once the immune system of the host is compromised. A high relapse rate caused by latent tubercle bacilli, for which no specific drug is currently available, remains a major obstacle in TB treatment. The key problems in TB eradication are the rise in incidence of multi drug-resistant/extensively drug-resistant Tuberculosis strains and incompatibilities between anti-TB and anti-HIV drugs [2]. There is an urgent need to develop new drugs that can kill drug-resistant Mtb in patients with latent TB infection.

Based on the current understanding, it is inevitable to develop novel drugs effective against the latent stage to contain the disease. Recently, it has been revealed that development of drug-resistance is not because of any inheritable genetic resistance mechanism but, it is conferred by changes in the physiological state of the mycobacteria [3]. However, more studies are needed to understand the metabolic processes that are critical for the pathogen to shift into dormancy, survive under this non-replicating drug-resistant state, and get reactivated when the immune system of the host is weakened. Consequently, the question whether the persistent state is controlled by the host immune system or if it is an intrinsic property of the bacteria themselves remains poorly understood.

A variety of factors that probably limit the bacterial growthin granulomas include oxygen and nutrient deprivation, acidic pH, and production of host factors such as nitric oxide [4-8]. Based on this, different dormancy models have been developed for better understanding of the mechanisms of dormancy and resuscitation that occur during infection within animal. In the *in vitro* model developed by Wayne, *M. tuberculosis* cells are subjected to gradual oxygen depletion. This results in the formation of an anaerobic, drug-resistant, non-replicating state [7]. Similar inactive non replicating state has been shown in nutrient starvation model of dormancy [9]. Under these conditions, the bacteria shut down protein synthesis and cell division but it restarts immediately after withdrawing the negative pressure. On the other hand, in *in vivo* Cornell model and non-culturable model developed by Shleeva and

Kaprelyants, bacteria remain non-culturable [10]; but the exact pathway which leads into the dormancy is still not clear. In all these models, the external environment of the bacilli is modified. But, the common internal targets that are getting modified because of change in the environment are still not fully explored.

Mycobacteria are bombarded with different reactive oxygen species (ROS) and reactive nitrogen species (RNS) inside the phagosomes of macrophages [11]. These ROS and RNS are in fact released to kill the pathogen; however, mycobacteria are not only resistant to this oxidative environment but preferably grow in high oxidative pressure. Together these studies set a precedence suggesting that oxidative stress induced by infection may be more detrimental to the host than facilitative in killing the bacteria [12]. How Mtb survives and grows in presence of different oxidative molecules is a subject of research that can lead us to understand the development of dormancy and resuscitation. Mtb has elaborate mechanisms to combat ROS and RNI produced by the host machinery. For example, superoxide can be detoxified by different superoxide dismutases (SODs) and catalase (KatG), peroxynitrite can be detoxified by KatG, and hydrogen peroxide can be detoxified by different thiol specific redox systems [13]. However, the role of this machinery under *in vitro* conditions remains unknown. Recently, we have shown that superoxide is actively produced by *M. smegmatis* which is linked to its growth (communicated).

Here, we show that blocking the endogenous superoxide production in bacteria leads to growth arrest and dormancy. Further, this dormancy is very similar to the non-cultivable type recently reported as well as observed in *in* vivo animal models and humans. Thus, our study gives a new insight into the development of dormancy and resuscitation in *M. smegmatis*.

3.2 Materials & Methods

3.2.1 Chemicals, Media, and Strains

All of the chemicals were purchased from Sigma-Aldrich, USA. The Dubos medium was purchased from DIFCO, USA. Zeil-Nielson Acid Fast Staining kit was purchased from Himedia, USA. *M. smegmatis* strain *mc²155* was a gift from AstraZeneca, India. Sub-culturing of the strain was routinely performed on Dubos albumin agar slants. The stock was maintained at -70ºC and sub-cultured once in liquid medium before inoculation in experimental culture medium. *M. smegmatis* culture was grown in 30 ml Dubos broth in a 100 ml flask incubated at 37°C on an orbital shaker set at 150 rpm. Mesa Green qPCR MasterMix Plus for SYBR® kit was purchased from Eurogentec.

3.2.2 Growth kinetics of *M. smegmatis* in presence of different inhibitors and modulators

The inhibitory activity of DPI against growing *M. smegmatis* was tested by following the method described earlier [14]. Briefly, 1% mid log phase culture of 1 OD_{620} was inoculated in 20ml of *M. phlei* medium in a 100 ml conical flask and allowed to incubate in aerobic condition with shaking at 150 rpm at 37°C within a shaker incubator (Thermo Electron Model No. 481; Thermo Electron Corp., Marietta, OH). The stock solution of compound in DMSO was added at different concentrations when cell OD reached to 0.4 and then its effect on growth was checked at different time points by reading the absorbance at 620 nm as well as by determining CFU/ml. In control, the same volume of the vehicle was added at the same time in the culture. In blank, all the components were added except the inoculums.

Pyrogallol was freshly prepared in autoclaved distilled water. This suspension was added at different time points after 24 hours of DPI (0.42mM) treatment and incubated at 37°C for different time points. The cell suspension was then plated on Dubos agar plates in triplicates. CFU/ml was determined after 3 days of incubating the agar plates within an incubator at 37°C. The control cells were not previously treated with DPI and in blank, cells were not added.

3.2.3 Determination of *M. smegmatis* viability

Viability testing was done by using a live dead Baclight staining kit by Molecular probes. It uses Propidium iodide to stain dead cells and Syto 9 to stain both live and dead cells. Briefly, 0.3 µl of 3.34mm Syto9 and 0.3 µl of 20mM Propidium iodide were added to 200µl of DPI treated culture and then observed under 600X magnification in fluorescent microscope (Evos, Life Technologies) after 5 minutes of incubation. The difference between the total number of cells and dead cells gives total number of viable cells. % Viable cells was calculated by using the formula {100-[[(C-T)/(C-B)]*100]}. Standardisation of both dyes and exposure time was done with respect to 100% live and 100% dead *M. smegmatis* cells. Dead cells were obtained by treating 0.4 OD_{620} of log phase *M. smegmatis*culture with 70% isopropanol for 2 hours [15].

3.2.4 Acid fast staining of *M. smegmatis* bacilli

Acid fast staining of the bacilli was done by following a method described earlier using a Ziehl-Neelsen (ZN) Acid fast staining kit from Himedia [16]. The stained cells were observed under a microscope (Nikon, Eclipse E200) with 400X magnification.

3.2.5 Scanning Electron Microscopy of *M. smegmatis* cells

Scanning electron microscopy of *M. smegmatis* cells was carried out by following an established protocol [17]. Briefly, mid-exponential growth phase cultures were diluted with Dubos medium to the cell density of 0.4 (620nm) and cells were fixed with 2.5% gluteraldehyde overnight at 4°C, washed four times for 15 minutes with sodium cocodylate buffer at room temperature and then post fixed with Osmium tetraoxide for 6 hours at 4°C. The samples were washed with sodium cocodylate buffer and dehydrated with graded ethanol series of 30%, 50%, 70% 90% ethanol for 30 minutes each and 95% and 100% ethanol for 1 hour each. Samples were then air dried and microscopy was performed with a Zeiss Supra 55VP (Oberkochen, Germany) microscope. Secondary electron images were taken at low electron energies between 2keV and 2.

3.2.6 RNA extraction and Real Time PCR of *M.smegmatis* genes

M. smegmatis cells were initially grown to 0.4 OD(620nm) to which DPI was added at 6μg/ml concentration. The extraction of total RNA from the bacilli was then carried out by adding 1 ml of sphaeroplast solution to 20 ml of culture and incubated overnight before total RNA was extracted by following an earlier method [18]. RNA (1μg/μl) was reverse transcribed to cDNA. The c-DNA samples were diluted to 10ng/μl and further amplification was carried out using gene-specific primers to generate SYBR Green-labelled PCR products using Mesa Green qPCR MasterMix Plus for SYBR® kit from Eurogentec on Eppendorf Mastercycler RealPlex. For all primers, the following temperature cycling profile was used: 5 min at 95°C followed by 30 seconds at 95°C and 30 seconds at annealing temperature for 40 cycles (Table 1). Transcript levels between various RNA samples were normalized using 23S rRNA. The experiments were carried out in triplicates for each time point.

Table 3.1: Details of the primers used in Real Time PCR study

Gene	Primer Sequence[a]		Amplification product size	Annealing Temperature [b]
	Forward primer	Reverse primer		
Msm23S	GAGCCCTCGACGTGTCGTGG	GCGCTTCACCCTGGCCATGG	201	58°C
Msmdevs	TGCTCGGTGTTCTGATCGAC	CCCGTTGGTCTTCTCGGTCA	176	60°C
Msmdevr	ATGCTCACCTCCTTCACGTC	GCGAAGTCTCTTCATCAGGG	177	60°C
MsmhspX	CGGCTGCCTCGGGTTCGGTC	GTGGTTACGAACTGCGCGCC	258	60°C

[a]Primers were designed using the IDTDNA primer designer software.

[b]Initial denaturation of 95°C for 2 minutes followed by 40 cycles of 94°C (30 seconds), respective annealing temp. (1 minute), 72°C (1 minute) and final extension of 10 minutes at 72°C were used for amplification of mentioned genes followed by melting curve analysis.

3.2.7 Detection of endogenous superoxide production by *Mycobacterium smegmatis*

Superoxide production by *M.smegmatis* was detected by following a modified HPLC-based method described earlier [19, 20]. Initially, 2 ml of 0.4 OD_{620} of *M. smegmatis* culture was taken washed by centrifugation at 10,000 rpm for 10 minutes at 4ºC and re-suspended in 1 ml of *M. Phlei* medium containing 100µM diethylene triamine pentaacetic acid (DTPA). DHE was added to it to achieve a final concentration of 25µM, mixed well and incubated at 37ºC for 90 minutes. After incubation, Triton X100 was added to the cell suspension at a concentration of 8%, vortexed for 5 minutes followed by sonication in a water bath sonicator (Bandelin Electronics, Germany) at 35kHz for 5 minutes. The cell lysate was filtered through 0.2µm membrane filter (Acrodisc 13 mm syringe filters, Pall Corporation) and then was taken for HPLC analysis (Waters). The chromatographic separation was performed on a C18 reverse phase column (Kinetex 5µ C18 100A, 250*4.60mm from Phenomenix). A gradient of solution A (1% formic acid in Water) and B (1% formic acid in ACN) was used as mobile phase at a flow rate of 0.4ml/min. Chromatographic runs were started with 100% solution A, decreased linearly to 60% solution A during the initial 10 minutes, continued at isocratic condition further for 15 minutes followed by a decrease to 0% solution A in the next 1minute, continued as 100% solution B for further 4 minutes. Solution A was then increased to 100% in next 1 minute continued this further for 9 minutes. 2-hydroxyethidium fluorescence was monitored by a fluorescence detector (excitation 480nm, emission 580nm) and DHE absorbance was monitored by a UV detector at 245nm. Area under the 2-hydroxyethidium peak was taken and concentration of 2-hydroxyethidium and ethidium was calculated by extrapolation from standard graph.

3.3 Results

3.3.1 Effect of blocking superoxide production on growth of *Mycobacterium smegmatis* cells

Our earlier studies have clearly shown that blocking superoxide production of *M.smegmatis* has also inhibited its growth. In order to clarify whether this growth inhibition is due to non-specific killing or because of possible development of dormancy, we first checked the dose response effect of DPI, an NADH oxidase inhibitor on the CFU of actively growing *M.smegmatis* bacilli. The growth of the bacilli on Dubos agar plate could possibly best reflect the number of healthy cells present at a given time period. It was observed that the CFU/ml decreases as concentration of DPI is increased when the effect was monitored after 24 hours of incubation (Figure 3.1A). Interestingly, the growth inhibition of bacilli was almost linearly increased with increase in DPI concentration. The dramatic increase in the growth inhibition may be due to increase in non-specific effect of DPI at higher concentration. To see effect of DPI with time, three concentrations of DPI were selected from Figure 3.1A namely, 2µg/ml, 6µg/ml and 10µg/ml. At 6µg/ml nearly 50% of the population was inhibited in 24 hours. Time kinetics data of 2µg/ml DPI shows that there is no significant inhibition of growth. In case of 10µg/ml DPI, growth was inhibited drastically within 24 hours only. But, in case of 6µg/ml DPI, growth was inhibited gradually (Figure 3.1B).

A) B)

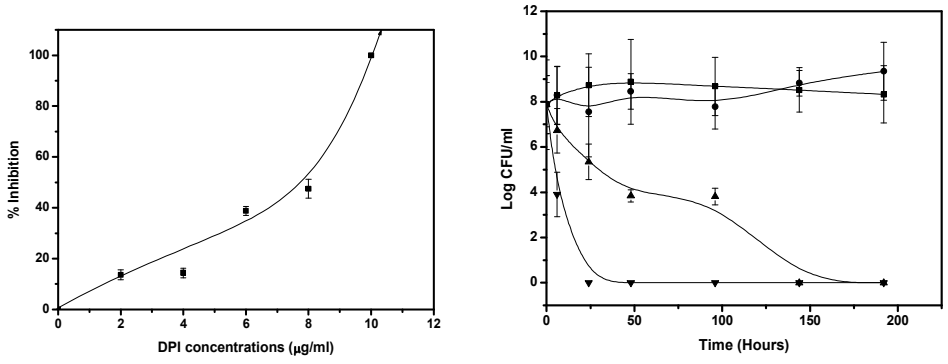


Figure 3.1: Growth of *Mycobacterium smegmatis* in presence of DPI (A) Effect of different concentrations of DPI on growth of *M. smegmatis*. A log phase culture of *M. smegmatis* bacilli at 0.4 OD_{620} was treated with different concentrations of DPI and incubated at 37°C for 24 hours before plating for CFU determination. % Inhibition was calculated using the formula {[(C-T)/(C-

B)*100]}. Cells having only vehicle served as control while in blank, no cells were added. (B) Kinetics of growth inhibition of *M. smegmatis* at different DPI concentrations. A log phase culture of *M. smegmatis* bacilli at 0.4 OD_{620} was treated with (■) vehicle control, (●) 2µg/ml, (▲) 6µg/ml and (▼) 10µg/ml of DPI. Rest of the experimental details are described in "Materials and Methods". All the samples were plated onto a Dubos agar plate for CFU/ml enumeration. % Inhibition was calculated using the formula {[(C-T)/(C-B)*100]}. Cells having only vehicle served as control while in blank, no cells were added. The results are shown as average of three identical experiments with S.D.

3.3.2 Viability of *M. smegmatis* after treatment with DPI

As DPI is inhibiting the growth of bacilli, it should be checked whether the cells are live and growth-restricted or dead. Therefore, viability of DPI treated cells was determined by using a Live-Dead kit which uses two fluorescent dyes namely, PI for dead cells and Syto 9 for total number of cells (Figure 3.2). Here, we selected the lowest concentration of DPI at 6µg/ml because the cells were found to linearly decrease with time. The results showed that within 24 hours of DPI treatment 97% cells remained viable. The number of viable cells was 98% within 48 hours and 90% within 72 hours. In 120 hours, the number remained 88% (Table 3.2). Thus, DPI is not killing the *M. smegmatis* cells. They are growth restricted and viable.

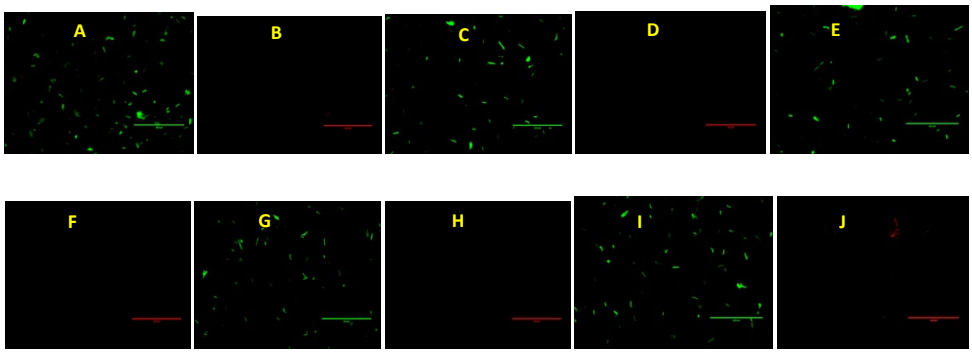


Figure 3.2: Live-Dead staining of DPI treated *Mycobacterium smegmatis* cells.(A) Microscopic pictures of *M. smegmatis* cells treated with 6µg/ml DPI for (A&B) 0hours, (C&D) 24hours, (E&F) 48hours, (G&H) 72hours and (I & J) 120hours. Green (A, C, E, G, I) pictures show total number of bacteria. Red (B,D,F,H,and J) pictures show dead bacteria. Multiple microscopic scans were captured for calculation.

Table 3.2: Quantitative estimation of % viable cells after DPI treatment

Duration of DPI treatment (Hours)	% Viable Cells[a]
0	100
24	97.23±0.23
48	98.18±0.43
72	90.27±0.87
120	88.28±0.15

[a]% Viable cells was calculated by using the formula {100-[[(C-T)/(C-B)]*100]} as described in the Materials and Methods. Results are average of three identical experiments with S.D.

3.3.3 Development of Dormancy in *Mycobacterium smegmatis* bacilli after treatment with DPI

As the cells were found to remain live even after 120 hours of treatment with DPI, it prompted us to check the development of dormancy in these cells. In order to characterize the development of dormancy, expression of DosS, DosR, and HspX genes were monitored [21]. The selective expression of the above mentioned genes was determined from the total RNA pool of the bacilli (Figure 3.3). Real time PCR results clearly indicated that the level of *dos*S, *dos*R, and *hsp*X transcripts in DPI treated samples was found to increase when monitored after 24 hours and 72 hours of treatment in comparison to the untreated control. The normalization of RNA was conducted by using total RNA with an equivalent amount of 23S rRNA.

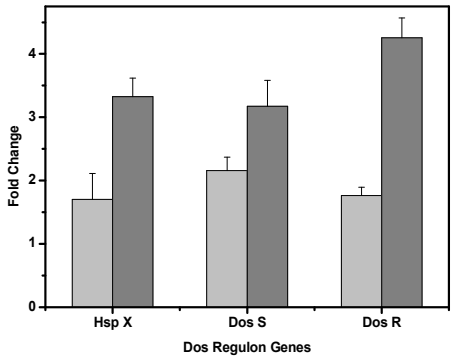


Figure 3.3: Effect of DPI on expression of DosRS regulon genes by Real Time PCR. Log phase 0.4 OD_{620} culture of *M. smegmatis* was treated with 6µg/ml of DPI for 24 hours (light gray) and 72 hours (dark gray). For each set of data, a control with

vehicle was run and 23S expression was used for normalization. The RNA extraction, c-DNA synthesis and Real time PCR procedures are described in "Materials & Methods". Results are average of three identical experiments with S.D.

3.3.4 Loss of Acid fast property of DPI treated bacilli

Loss of acid fast property is one of the major characteristic changes that happen when mycobacteria shift from active stage to dormant stage [3]. A very common and classical method to discriminate between acid fast and non-acid fast bacilli is staining by Ziehl Neelsen's stain. We applied this method to see acid fast property of *M. smegmatis* cells treated with DPI (Figure 3.4). Control cells without any treatment with DPI showed pink-purple coloured acid fast bacilli. Whereas, there was decrease in acid fast property of DPI treated cells after 24 hours of treatment which continued till 120 hours. It should also be noted that the colour of the stain observed in cells after 24 hours of treatment did not remain same in 72 hours and 120 hours respectively. The results also indicated that probably the membrane composition of these cells is different.

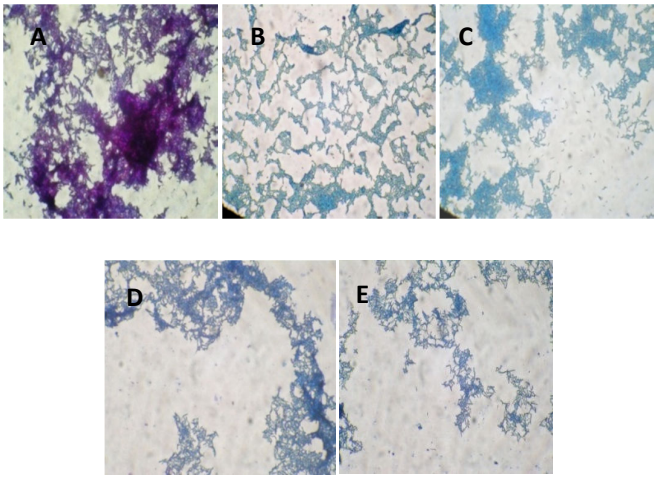


Figure 3.4: Acid fast staining of DPI treated *M. smegmatis* bacilli. Bright field images of Log phase 0.4 OD_{620} culture of *M. smegmatis* treated with 6µg/ml of DPI for (A) Vehicle control, (B) positive control (Wayne model), (C) 24 hours, (D) 72 hours and (E) 120 hours. Images are taken under 400X magnification.

3.3.5 Determination of the cell size of DPI treated *Mycobacterium smegmatis* bacilli

Earlier reports suggested that reduction in cell size is another characteristic feature observed during shift of actively growing bacilli to the dormant stage [22]. DPI treated cells were subjected to scanning electron microscopy to precisely observe the changes in cell shape/size (Figure 3.5). The treated cells were found shorter in length in comparison to untreated cells. Normally, mycobacterial cell length varies between 3-5 µm but DPI treated cell length was found to be around 1-3 µm. Within 24 hours of DPI treatment, 77% cells were found to be in range between 1-3 µm. The number increased to 96% till 120 hours. Wayne model culture which was used as a positive control for dormancy showed 85% cells reduced in size (Table 3.3). This observation again strengthened the earlier observations indicating development of dormancy in *M. smegmatis* cells treated with DPI.

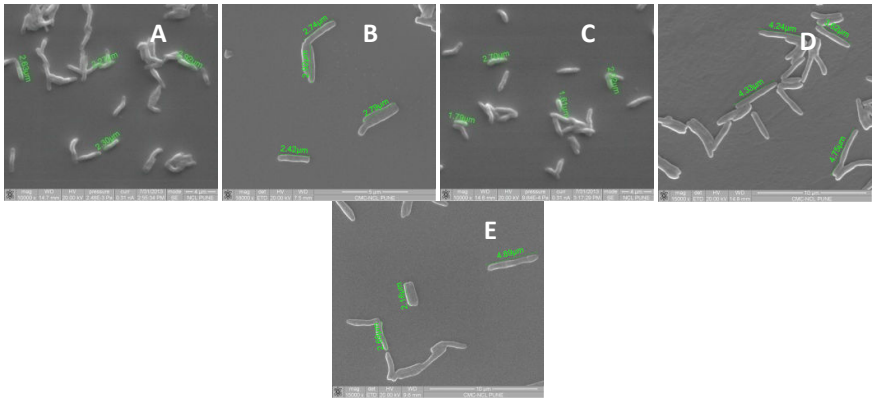


Figure 3.5: Size Determination of DPI treated dormant bacilli. (A) Scanning electron microscopic images of Log phase 0.4 OD_{620} culture of *M. smegmatis* treated with 6µg/ml of DPI for (A) 24 hours, (B) 72 hours (C) 120 hours, (D) Vehicle control (E) Positive control (Wayne model). Samples were prepared as described in "Materials and Methods". The images are representative of three separate experiments carried out under identical conditions.

Table 3.3: Number of DPI treated *M. smegmatis* cells reduced in size.

Samples	% of cells reduced in size (1-3µm)
Control	7%
DPI 24 hours	77%
DPI 72 hours	89%
DPI 120 hours	96%
Wayne Model	85%

Cells were counted and divided in ranges between 1-3µm (reduced) and 3.1-5µm (Normal). % of cells reduced in size was calculated using the formula {[(C-T)/(C-B)]*100}. C: Number of cells not reduced in size in control, T: Number of cells not reduced in size in treated. Multiple microscopic images were taken to count the cells. Experiment was done in triplicates.

3.3.6 Development of antibiotic resistance in DPI induced dormant *M. smegmatis* cells

Resistance to antibiotics is a hallmark of Latency in mycobacteria [4,23-26].We also found that when *M. smegmatis* cells were treated with DPI for 24 hours, developed resistance against the anti-tubercular drugs namely, Rifampicin, Isoniazid, Streptomycin and Pyrazinamide (Table 3.4).

Table 3.4: Development of resistance against anti-tubercular drugs

Antibiotics		% Inhibition[b]	
		After 24 hours	After 48 hours
Rifampicin	0.5 µg/ml[a]	70.30±0.52	77.19±0.23
Isoniazid	1 µg/ml[a]	91.76±0.81	95.28±0.45
Streptomycin	0.25 µg/ml[a]	97.98±1.02	98.12±0.75
Pyrizinamide	10 µg/ml[a]	85.23±0.43	91.83±0.11

[a]Drugs were added at their respective MIC values on *M. smegmatis* taken from Singh et al, (84) 2011, Journal of Microbiological Methods, page no.206.

[b]Viable bacilli were enumerated by live dead staining method and compared with control not subjected to antibiotic treatment.% Inhibition was calculated using the formula {100-[[(C-T)/(C-B)]*100]}.

M. smegmatis cells were incubated with DPI (6µg/ml) for 24 hours. After which drugs were added and further incubation was done for another 48 hours. After 24 hours and 48 hours of antibiotic treatment samples were prepared for live dead staining as described in Materials and Methods. Multiple microscopic scans were captured. Results are average of three identical experiments with S.D.

3.3.7 Resuscitation of DPI treated dormant cells

The experimental data so far have established the fact that addition of DPI in a 0.4 OD_{620} culture (~$8x10^7$cells/ml) transformed actively growing bacilli into non-replicating stage within 24 hours, which persisted beyond 120 hours of incubation. Inhibition of endogenous production of superoxide by DPI could be considered as the most prominent explanation for the observed dormancy phenotype in *M. smegmatis* cells. This dormant culture should be resuscitated by extraneous addition of superoxide if superoxide deprival is the major reason. In order to validate this hypothesis, 0.42mM pyrogallol was added to dormant cultures, which reactivated cells to the replicating state, as evident by the two log increase in cell number within 36 hours (Figure 3.6A). Pyrogallol, a known producer of superoxide has been used in several experiments to generate superoxide [27-29]. Apart from resuscitation of the dormant cells, we checked the level of superoxide production in pyrogallol treated culture (Figure 3.6B). DPI treated (24 hours) dormant cells were incubated with 0.42mM pyrogallol for 1, 6 and 24 hours and then incubated with DHE for 90 minutes before monitoring production of2-hydroxyethidium and ethidium by HPLC. There was an increase in 2-hydroxyethidium levels by 115%, 161% and 218% as compared to control (control is 100%) in 1 hour, 6 hours and 24 hours of pyrogallol treated samples respectively. This result clearly indicates that increase in superoxide level in the DPI treated dormant cells is directly linked to resuscitation of the *M. smegmatis* bacilli. The ethidium level was found to decrease in 1 hour and then was unaffected till 24 hours of pyrogallol treatment. Chromatographic separation of 2-hydroxyethidium and ethidium profile and the standard graph is given in Chapter 2.

(A) (B)

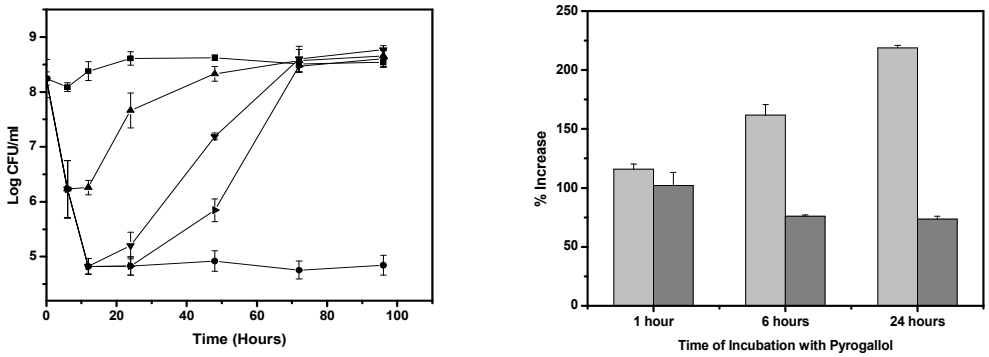


Figure 3.6: Resuscitation of DPI induced dormant *Mycobacterium smegmatis* bacilli by superoxide.(A) Effect of Pyrogallol on the number of cultivable cell count in DPI treated *M. smegmatis* cells. Resuscitation kinetics of (●) DPI 6µg/ml, (▲) Pyrogallol (0.42mM) added after 6 hours of DPI treatment, (▼) Pyrogallol (0.42mM) added after 12 hours of DPI treatment (►) Pyrogallol (0.42mM) added after 24 hours of DPI treatment and a (■) Vehicle control. (B) Effect of Pyrogallol on superoxide production in DPI treated *M. smegmatis* cells. Pyrogallol (0.42mM) was added after 24 hours of DPI treatment and incubated for 1 hour, 6 hours and 24 hours as shown in the figure. Oxyethidium (Light gray bars) and Ethidium (Dark gray bars) levels were found by performing DHE HPLC method as provided in Material & Methods. Results are average of three identical experiments with S.D.

3.4 Discussion

One of the most challenging questions in TB eradication has been identifying the factors involved in converting replicating (active) to non-replicating (dormant) state of *Mycobacterium tuberculosis* bacilli as well as its reactivation. *In vitro* dormancy models were developed to explain some of the phenotypic and metabolic features observed in clinical samples [30]. But, the mechanism by which this conversion takes place is still unknown. Here, we have used *M. smegmatis* as a model system because *M. smegmatis* bacilli follow most of the key features of dormancy seen inthe virulent Mtb, together with having the advantage of being a non-pathogenic and fast growing organism [14, 21, 26, 31-33].

It was earlier reported that ascorbic acid, a known antioxidant, induced dormancy in *M.tuberculosis* [34]. The mechanism was suggested to be the depletion of level of O_2 within the culture as it happens in Wayne culture. Our earlier finding clearly suggested that *M. smegmatis* is not only capable of producing significant extent of superoxide (Chapter 2), its decrease within the cell by adding antioxidants as well as modulators could lead to growth inhibition (Data not shown here). To validate our assumption that antioxidants induce dormancy by scavenging superoxide, DPI was selected instead of ascorbic acid because DPI is an inhibitor of NADH oxidase enzyme which is supposed to be a major contributor of superoxide produced in *M. smegmatis*(Chapter 2) and it does not have wide ranging effects on other ROS molecules like ascorbic acid. Addition of DPI in the *M. smegmatis* culture induced bacteriostasis characteristic of dormancy supported by the induction of the DosRS regulon genes, decrease in cell size, change in acid fast staining property and resistance to antibiotics (Figures3.1,3.3,3.4,3.5 and Tables 3.3,3.4). DosR is widely considered essential for TB latency as apparent from the name DosR, Dormancy survival Regulator [1]. Remarkably, Mtb DosS acts as a redox sensor and responds to reduction in the electron transport system [35].The direct link between superoxide withdrawal and induction of dormancy in the bacilli was further confirmed by resuscitation of the dormant bacilli by addition of exogenous superoxide using pyrogallol, a superoxide generator as well as its confirmation with the help of DHE (Figure3.6). Pyrogallol is a known superoxide generator and is often used to investigate the role of superoxide in biological systems [36-38]. This observation

proved that inter-conversion of active and dormant cells is dependent on the level of the superoxide within the bacterium. Our result also suggests that development of dormancy in mycobacterium cells after treatment with an antioxidant like ascorbic acid probably happens by following similar mechanisms due to lowering of superoxide level within the cells.

DPI is a known and often considered as a specific inhibitor of NADH and NADPH oxidases [39, 40]. But, there are many questions raised about the specificity of DPI since its other functions in the cell are coming up. It was shown to inhibit mitochondrial NADH dehydrogenase [41]. In Trypanosoma brucei, it inhibits rotenone insensitive NADH dehydrogenase dependent superoxide production [42]. Apart from its inhibitory activity against NADH oxidases and dual oxidases, it also acts as an iodide specific transporter in thyroid cells of pig [43]. Although it is not clear whether it is a specific inhibitor of NADH oxidase or not, its role in inhibiting superoxide production in the cell cannot be denied and is also apparent from our earlier data (chapter 2).

Of note, non-cultivability, which is an important characteristic of clinical samples, was not observed in other *in vitro* models, such as Wayne's hypoxia and nutrient starvation, but was well achieved in DPI induced dormant cultures (Figure 3.1). The rapid non-cultivability and dormancy phenotype in DPI treated *M. smegmatis* bacilli could characteristically make it as most favourable dormancy model very similar to the cells obtained from clinical samples. This finding could be useful in designing new in vitro and in vivo models for studying dormancy and screening of potential dormancy specific inhibitors. Major advantage of using DPI induced dormant culture lies in the fact that large amount of non-cultivable cells could be obtained within short period of time compared to any other dormancy models.

In summary, this study suggests that a decrease in the intracellular ROS levels through inhibition of superoxide production could convert the bacilli from the active to dormant form or vice versa. So, DPI induced dormancy could be placed as a new dormancy model for studying mycobacteria under in vitro conditions. This finding also provides a plausible molecular mechanism for the development of latency as well as reactivation of tuberculosis in humans.

3.5 References

1. Rustad T.R., Sherrid A.M., Minch K.J. and Sherman D.R., Hypoxia: a window into *Mycobacterium tuberculosis* latency, Cellular Microbiology, 11:1151-1159, 2009.

2. Gupta A. and Bhakta S., An integrated surrogate model for screening of drugs against *Mycobacterium tuberculosis*, J. Antimicrob. Chemotherapy, 67:1380–1391, 2012.

3. Deb C., Lee C., Dubey V.S., Daniel J., Abomoelak B., Sirakova T.D., Pawar S., Rogers L. and Kolattukudy P.E., A novel *in vitro* multiple-stress dormancy model for *Mycobacterium tuberculosis* generates a lipid-loaded, drug-tolerant, dormant pathogen, PLoS ONE, 4:1-15, 2009.

4. Betts J.C., Lukey P.T., Robb L.C., McAdam R.A. and Duncan K., Evaluation of anutrient starvation model of Mycobacterium tuberculosis persistence by gene and protein expression profiling, Mol Microbial., 43:717–731, 2002.

5. Fisher M.A., Plikaytis B.B. and Shinnick T.M., Microarray analysis of the Mycobacterium tuberculosis transcriptional response to the acidic conditions found in phagosomes, J Bacteriol., 184:4025–4032, 2002.

6. Voskuil M.I., Mycobacterium tuberculosis gene expression during environmental conditions associated with latency, Tuberculosis (Edinb), 84:138–143, 2004.

7. Wayne L.G. and Hayes L.G., An in vitro model for sequential study of shiftdownof Mycobacterium tuberculosis through two stages of nonreplicating persistence, Infect Immun 64: 2062–2069, 1996.

8.Voskuil M.I., Schnappinger D., Visconti K.C., Harrell M.I., Dolganov G.M., et al., Inhibition of respiration by nitric oxide induces a Mycobacterium tuberculosis dormancy program, J Exp Med., 198: 705–713, 2003.

9. Gengenbacher M., Rao S.P.S., Pethe K. and Dick T., Nutrient-starved, non-replicating Mycobacterium tuberculosis requires respiration, ATP synthase and isocitrate lyase for maintenance of ATP homeostasis and viability, Microbiology, 156:81-87, 2010.

10. Shleeva M., Mukamolova G.V., Young M., Williams H.D. and Kaprelyants A.S., Formation of 'non-culturable' cells of Mycobacterium smegmatis in stationary phase in response to growth under suboptimal conditions and their Rpf-mediated resuscitation, Microbiology, 150:1687-1697, 2004.

11. Meylan P.R., Richman D.D. and Kornbluth R.S., Reduced intracellular growth of mycobacteria in human macrophages cultivated at physiologic oxygen pressure, Am. Rev. Respir. Dis., 145:947–953, 1992.

12. Oberley-Deegan R.E., Rebits B.W., Weaver M.R., Tollefson A.K., Bai X., McGibney M., Ovrutsky A.R., Chan E.D. and Crapo J.D., An oxidative environment promotes growth of *Mycobacterium abscessus,* Free Radic. Biol. and Med., 49:1666–1673; 2010.

13. Ng V.H., Cox J.S., Sousa A.O., MacMicking J.D. and McKinney J.D., Role of KatG catalase-peroxidase in mycobacterial pathogenesis: countering the phagocyte oxidative burst, Mol. Microbiol., 52: 1291–1302; 2004.

14. Khan A., Akhtar S., Ahmad J.N. and Sarkar D., Presence of a functional nitrate assimilation pathway in *Mycobacterium smegmatis,* Microb. Pathog., 44:71-77; 2008.

15. Taskin B., Gozen A.G. and Duran M., Selective Quantification of Viable *Escherichia coli* Bacteria in Biosolids by Quantitative PCR with Propidium Monoazide Modification, Applied And Environmental Microbiology, 77:4329–4335, 2011.

16. Gang Zhao et al, A Highly Efficient Ziehl-Neelsen Stain: Identifying De Novo Intracellular Mycobacterium tuberculosis and Improving Detection of Extracellular M. tuberculosis in Cerebrospinal Fluid, *J. Clin. Microbial.*, 50:1166, 2012.

17. Takayama K., Wang L. and Merkal R.S., Scanning Electron Microscopy of the H37Ra Strain of *Mycobacterium tuberculosis* Exposed to Isoniazid, Antimicrob Agents Chemother., 4: 62–65, 1973.

18. Akhtar S., Sarkar S., Mishra A. and Sarkar D., A method to extract intact and pure RNA from mycobacteria, Analytical Biochemistry, 286–288, 2011.

19. Zhao H., Kalivendi S., Zhang H.,JosephJ., Nithipatikom K., V´Asquez-VivarJ. and Kalyanaraman B., Superoxide reacts with hydroethidine but forms a fluorescent product that is distinctly different from ethidium: potential implications in intracellular fluorescence detection of superoxide,Free Radic. Biol. and Med. 34:1359–1368, 2003.

20. Laurindo F.R.M., Fernandes D.C. and Santos C.X.C., Assessment of Superoxide Productionand NADPH Oxidase Activity by HPLC Analysis of DihydroethidiumOxidation Products, Methods in Enzymology, 441:238-260, 2008.

21. Bagchi, G. B.; Das, T. K.; Tyagi, J. S. Molecular analysis of the dormancy response in *Mycobacterium smegmatis*: expression analysis of genes encoding the DevR-DevS two component system, Rv3134c and chaperone K-crystallin homologues. FEMS MicrobioLett.211:231-237; 2002.

22. Salina E.G., Zhogina Y.A., Shleeva M.O., Sorokoumova G.M., Selishcheva A.A. and Kapreylants A.S., Biochemical and Morphological Changes in Dormant ("Nonculturable") *Mycobacterium smegmatis* Cells, Biochemistry (Moscow), 75: 72-80, 2010.

23. Gomez J.E. and McKinney J.D. M., Tuberculosis persistence, latency, and drug tolerance. Tuberculosis (Edinb), 84: 29–44, 2004.

24. Haapanen J.H., Kass I., Gensini G. and Middlebrook G., Studies on the gaseous content of tuberculous cavities, Am Rev Respir Dis 80: 1–5, 1959.

25. Dannenberg A.M. and Tomashefski J.F., Pulmonary diseases and disorders. InPathogenesis of pulmonary tuberculosis. Fishman AP, ed. New York, NY: McGraw-Hill, 1997.

26. Wayne L.G. and Sohaskey C.D., Nonreplicating persistence of Mycobacterium tuberculosis, Annu Rev Microbiol., 55: 139–163, 2001.

27. Yamada J., Yoshimura S., Yamakawa H., Sawada M., Nakagawa M., Hara S., Kaku Y., Iwama T., Naganawa T., Banno Y., Nakashima S. and Sakai N., Cell permeable ROS scavengers, Tiron and Tempol, rescue PC12 cell death caused by pyrogallol or hypoxia/reoxygenation, Neuroscience Research, 45:1-8, 2003.

28. Han Y.H., Kim S.Z., Kim S.H. and Park W.H., Pyrogallol as a glutathione depletorinduces apoptosis in HeLa cells, International Journal of Molecular Medicine, 21:721-730, 2008.

29. He D., Ma X., Chen Y., Cai Y., Xiaochen R., Bruce I.C., Xia Q., Shi G. and Jin J., Luteolin inhibits pyrogallol-induced apoptosis through the extracellular signal-regulated kinase signalling pathway, FEBS Journal, 279:1834–1843, 2012.

30. Shleeva M. O. et al., Formation and resuscitation of non-culturable cells of *Rhodococcus rhodocrous* and *Mycobacterium tuberculosis* in prolonged stationary phase, Microbiology, 148:1581–1591, 2002.

31. Khan A. and Sarkar D., Identification of a respiratory-type nitrate reductase and its role for survival of *Mycobacterium smegmatis* in Wayne model, Microbial Pathogenesis, 41: 90-95, 2006.

32. Dick T., Lee B.H. and Murugasu O.B., Oxygen depletion induced dormancy in *Mycobacterium smegmatis*, FEMS Micro bio Lett, 163:159-164,1998.

33. Anuchin A.M. et al, Dormant forms of *Mycobacterium smegmatis* with distinct morphology, Microbiology, 155:1071-9, 2009.

34. Taneja N.K., Dhingra S., Mittal A., Naresh M. and Tyagi J.S., Mycobacterium tuberculosis Transcriptional Adaptation, Growth Arrest and Dormancy Phenotype Development Is Triggered by Vitamin C, PLoS ONE, 5:e10860, 2010.

35. Honaker R.W., Dhiman R. K., Narayanswamy P., Crick D. and Voskil M. I., DosS responds to a reduced electron transport system to induce the *Mycobacterium tuberculosis* DosR Regulon, J. Bact, 192:6447–6455, 2010.

36. Yamada J., Yoshimura S., Yamakawa H., Sawada M., Nakagawa M., Hara S., Kaku Y., Iwama T., Naganawa T., Banno Y., Nakashima S. and Sakai N., Cell permeable ROS scavengers, Tiron and Tempol, rescue PC12 cell death caused by pyrogallol or hypoxia/reoxygenation, Neuroscience Research, 45:1-8, 2003.

37. Han Y.H., Kim S.Z., Kim S.H. and Park W.H., Pyrogallol as a glutathione depletor induces apoptosis in HeLa cells, International Journal of Molecular Medicine, 21:721-730, 2008.

38. He D., Ma X., Chen Y., Cai Y., Ru X., Bruce I.C., Xia Q., Shi G. and Jin J., Luteolin inhibits pyrogallol-induced apoptosis through the extracellular signal-regulated kinase signalling pathway, FEBS Journal, 279:1834-1843, 2012.

39. Li N., Yi F., Spurrier J.L. Bobrowitz C.A. and Zou A., Production of superoxide through NADH oxidase in thick ascending limb of Henle's loop in rat kidney, Am. J. Physiol. renal physiol, 282:f1111-f1119, 2002.

40. Ellis J.A., Mayer S.J. and Jones O.T.G., The effect of NADPH oxidase inhibitor diphenelyleneiodonium on aerobic and anaerobic microbicidal activities of human neutrophils, Biochem. J., 251:887-891, 1988.

41. O'Donnell V.B., Tew D.G., Jones O.T.G. and England P.J., Studies on the inhibitory mechanism of iodonium compounds with special reference to neutrophil NADPH oxidase, Biochem J., 290:41-49,1993.

42. Fang J. and Beattie S., Rotenone-insensitive NADH dehydrogenase is a potential source of superoxide in procyclic Trypanosoma brucei mitochondria, Molecular & Biochemical Parasitology, 123:135-142, 2002.

43. Massart C., Guisti N., Beauwens R., Dumont J.E., Milot F. and Sande J.V., Diphenyleneiodonium, an inhibitor of NOXes and DUOXes, is also an iodide-specific transporter, FEBS Open Bio, 4:55-59, 2014.

CHAPTER 4

Gene and Protein Expression Profiling of DPI Induced Dormancy Model in *Mycobacterium smegmatis*

4.1 Introduction

Antimycobacterial chemo-therapy to cure a patient of tuberculosis takes at least 6 months because the available drugs are poorly active against the persistent form of Mtb. New drugs with potent inhibitory activity areurgently needed to shorten the duration of drugtreatment and reduce the risk of relapse [1, 2].

It is very essential to understand the changes that take place when active form is converted to dormant form. Currently available models are not able to explain all the characteristics of latent tubercular bacilli. There is an urgent need to develop new models of dormancy which will not only represent the latent tuberculosis better but also provide novel biomarkers to use in developing screening protocols for drug discovery under *in vitro* and *in vivo* conditions.

We have seen that DPI induces dormancy phenotype in *M. smegmatis*. It is already established that DPI induced dormancy provides non-cultivable cells and that too under aerobic conditions. How the bacilli transform into the dormancy form is still a subject of research though our findings clearly indicate that lack of superoxide production is possibly the main reason. Although there is strong possibility of significant decrease in superoxide production within the granulomas in infected lung tissues due to anaerobic environment, it needs a proof. Meanwhile, the DPI induced dormant bacilli may further be characterized and compared with known as well as established dormancy models at transcriptional and translational level as well to find out new genes/proteins which are exclusively up regulated in DPI induced dormancy model to match with the latent bacilli under in vivo conditions. In order to carry out this, 4 samples were taken namely, aerobically growing *M. smegmatis* which served as a control (to which all other models were compared), Wayne's gradual oxygen depletion model (a known model used to study dormancy in mycobacteria) [3, 4], DPI treated *M. smegmatis* cells and ascorbic acid (AA) treated *M. smegmatis* cells. Ascorbic acid mimics multiple intracellular stresses and has wide-ranging regulatory effects on gene expression and physiology of Mtb which leads to growth arrest and a ‘dormant’ drug-tolerant phenotype [5].

There are severalreports on the proteomes of mycobacterial strains where cytosolic as well as cell wall proteins and culturefiltrate proteins, have been

identified[6-13]. Many stress proteins like HspX, Sigma factors are up-regulated during hypoxia in Mtb [14]. Alanine dehydrogenase has been identified as an antigen produced by virulent Mtb. It is one of the few antigens with enzymic properties thatmake it attractive for diagnostic and therapeutic interventions.The activity ofthe enzyme increases during dormancy development in*M. smegmatis*[15]. As suggested in literature, the functionof this enzyme is the generation of alanine for protein andpeptidoglycan synthesis. In dormancy, protein as well as peptidoglycan synthesis are down regulated. Therefore, it is possible that this enzyme might support the maintenanceof the NAD^+ pool under dormancy as alanine synthesis is coupled toNADH oxidation [15]. Gene expression profile of nutrient starved *M.tuberculosis* and *M. smegmatis*has shown many important genes that are deferentially expressed in dormant culture. One of them is RelA which maintains the pool of an unusual nucleotide, ppGpp, accumulation of which is a hallmark of stringency [16]. The gene encoding isocitrate lyase (icl) is shown to be up regulated in dormancy models like multiple stress dormancy model, Wayne's in vitro model and nutrient starvation model [17,18]. Icl is required for the virulence and persistence in Mtb in murine macrophages [19]. It is required for survival of bacteria in activated macrophages but not in resting macrophages [20]. Also, it is essential for survival of Mtb in the lungs of mice during the persistent phase of infection [21]. To sustain in a nutrient starved condition, it diverts carbon from fatty acid metabolism to glyoxylate pathway [22].

Thus characterization of DPI induced dormancy model by gene expression profile and protein expression profile would lead us to understand the dormancy in a better way and help us to identify new targets for the dormant stage of mycobacteria. This study will also help us to understand the molecular mechanisms that are important for achieving dormancy.

4.2 Materials & Methods

4.2.1 Samples used for Microarray and LC-MS^E studies

Four samples of *M. smegmatis*were used namely, aerobically growing cells (Log phase culture of 1 OD_{620}) which served as a control, DPI treated cells (0.4 OD_{620}culture treated with DPI at a final concentration of 6µg/ml for 24 hours), and Ascorbic acid treated cells and Wayne Model cells (6^{th} day culture, NRP-2 stage). Preparation of DPI treated cells, Wayne model and aerobically growing *M. smegmatis* cells are described in detail in chapter 3. *M. smegmatis* cells were grown till 0.4 OD_{620} is achieved to which ascorbic acid at a final concentration of 2.5mM was added and incubated at 37°C for 24 hours.

4.2.2 Microarray studies (Gene Expression Profiling)

We outsourced the services of Genotypic Technology Pvt. Ltd. Bangalore India, for gene expression profiling (microarray studies).

RNA was used as a starting material.50 ng/µl RNA was used for microarray studies. RNA was converted into c-DNA and then to c-RNA with Cy3 labelled probe using Agilent's Quick-Amp labelling Kit. Random hexamer method of labelling was done followed by T7 promoter based-linear amplification to generate labelled complementary RNA (One-Colour Microarray-Based Gene Expression Analysis). 600ng of c-RNA was used for one array. For total RNA and c-RNA Purification Qiagen's RNeasy minikit (Cat#74104) was used. For Hybridization, Agilent's In situ Hybridization kit (5188-5242) was used. RNA quality was checked by using Bioanalyzer. Probes were spotted on glass slides (8 arrays on one glass slide; one array has 60k probes). After hybridization, slides were scanned using Agilent's Microarray scanner. Using feature extraction software raw data files were created which were then used in Genespring GX 12.0 software for normalization of data by Percentile Shift Normalization method.

4.2.3 Preparation of crude protein extract of *M. smegmatis* for whole cell proteome analysis

The proteomic experiment and analysis was carried out by modifying an earlier described method [23]. Approximately, 25ml cells of each type of culture were harvested by centrifugation at 9000 RPM for 30min. The pellet was re-suspended in 2% Rapigest (1 vial diluted in 500µL 50mM Ammonium Bicarbonate (ABC)). After re-solubilisation, sonication was done at 75 Hz with 30 second pulse with a break of 45 second. This cycle was repeated for eight times. After sonication samples were centrifuged for 10 minutes at 12000rpm and the supernatant containing proteins was concentrated to 10-20 µl using MW 3000 cut off spin column. The concentrate was used for determining protein concentration by Bradford's method.

4.2.4 Trypsin digestion of *M. smegmatis* crude extract

Approximately, 50µg of crude protein mixture was heated to 80°C for 15 minutes and then 3 µL of 100mM DTT (made up in 50mM ABC) was added before heating at 60°C for another 15 minutes. Then, 3 µL of 200mM Iodoacetamide (made up in 50mM ABC) was added and the solution was incubated at room temperature for 30 minutes in dark. 1µl of 1 µg/µl trypsin (prepared in 50mM ABC) was added and the solution was incubated at 37°C for overnight. 1 µl of concentrated HCl was added and the solution was incubated at 37°C for 20 minutes and centrifuged at 30000 RPM for 30minutes. The supernatant was collected and passed through a MW 5000 spin cut off column and transferred to a clean eppendorf tube to store it at -80°C.

4.2.5 LC/MS^E Analysis

For LC/MS^E an earlier method was used with modifications [23]. Briefly, prior to LC/MS^E analysis, each digested protein sample was spiked with a predigested bovine serum albumin internal standard (Waters, Milford, MA) at a level of 50 fmol per 10 µl injection. For sample analysis, 10 µl aliquots of proteome tryptic digests were analyzed in duplicate (2 technical replicates per sample) by LC/MS^E using a nano ACQUITY ultra pressure liquid chromatography (UPLC) and Premier Q-Tof mass spectrometer equipped with a nanolock spray ion source (Waters). Samples were injected online onto a Waters Symmetry C18 trapping cartridge (300 µm i.d. × 1 cm length) at a flow rate of 10µl/min. Next, peptides were separated by in-line gradient

elution onto a 75 μm i.d. × 25 cm column packed with BEH C18 Stationary phase (Waters), 1.7 μm particle size, at a flow rate of 300 nL/min using a linear gradient from 2 to 40% B over 120 min (A = 0.1% formic acid in water, B = 0.1% formic acid in acetonitrile). The Q-Tof was operated in the LC/MS^E mode of acquisition, where alternating 2 s scans of low (4 V) or high (10−32 V) collision energies are used to generate either intact peptide ions (low energy) or peptide product ions (high energy). Glu-fibrino peptide at a concentration of 200fmol/μl (*m*/*z* 785.8426) was infused via the nano lock spray ion source at a flow rate of 600nL/min and sampled every 30 s as the external mass calibrant. Samples were injected as sets based on treatment from earliest to latest time points. A protein standard (tryptic digest of bovine serum albumin) analyzed prior to the first sample injection and again following the last sample injection showed no significant loss in instrument sensitivity or performance during the course of the analysis.

4.2.6 Data Processing and Database Searching

Each raw data file was processed using Protein Lynx Global Server V 2.2.5 software (Waters) to generate charge state reduced and isotope free precursor mass lists as well as associated product ion mass lists for subsequent protein identification and quantification. Each processed file was then searched against the Uniprot protein database (http://www.uniprot.org/) using the IDENTITY^E database search algorithm within PLGS 2.2.5. Prior to searching, the internal standard bovine serum albumin sequence was added to the database and redundant entries (identical sequences reported more than once) were removed using an *ad hoc* C++ program. Except for the false positive rate, default search parameters were used including the "automatic" setting for mass accuracy (10 ppm for precursor ions and 15 ppm for product ions), a minimum of 1 peptide match per protein, a minimum of 3 consecutive product ion matches per protein, and a minimum of 7 total product ion matches per protein. The maximum false positive rate (FPR) against the randomized forward database was set to 2%, and the absolute protein quantification functionality was enabled using the bovine serum albumin. Only 1 missed tryptic cleavage site was allowed during the search. A fixed carbamidomethyl-Cys modification was used, in addition to the following variable modifications: deamidation of Asn and Gln; oxidation of Met; and dehydration of Ser and Thr (in-source modification).

4.3 Results

To study the gene expression profile of *M. smegmatis*under different dormancy conditions, we availed the services of Genotypic India, Pvt. Ltd. For this, the above mentioned 4 samples were used. Genes were considered to be significantly differentially expressed compared with the aerobic control if they displayed at least a 1.5 fold induction or repression.

4.3.1 Global transcriptome analysis of active and dormant *M. smegmatis* bacilli

In Wayne model, total of 4490 number of genes were detected out of which 2705 correspond to coding genes and the rest correspond to non coding genes. Amongst the coding genes, 1204 were up-regulated and 1501 were down-regulated. In DPI treated sample, total of 5488 number of genes were detected, out of which, 3235 correspond to coding genes and the rest correspond to non coding genes. Amongst the coding genes, 1499 were up-regulated and 1736 were down-regulated. In ascorbic acid treated sample, total of 4193 number of genes were detected, out of which, 2470 correspond to coding genes and the rest correspond to non coding genes. Amongst the coding genes, 1019 were up-regulated and 1451 were down-regulated (Table 4.1).

Table 4.1: Summary of Differentially Regulated Genes compared to Aerobic (Active)

Samples	Up (Total/Coding)	Down (Total/Coding)
Hypoxia Wayne model	2458/1204	2032/1501
DPI	3029/1499	2459/1736
Ascorbic acid	2277/1019	1916/1451

Up-regulation and down-regulation values are calculated with respect to control (aerobic sample).

4.3.1.1 Statistical validation using Scatter Plot based on Signal Intensities

The scatter plots show the relationship between two sets of data (Figure 4.1). The pattern clearly indicated that cells obtained from all three cultures are very similar and their expression profiles are following similar pattern.

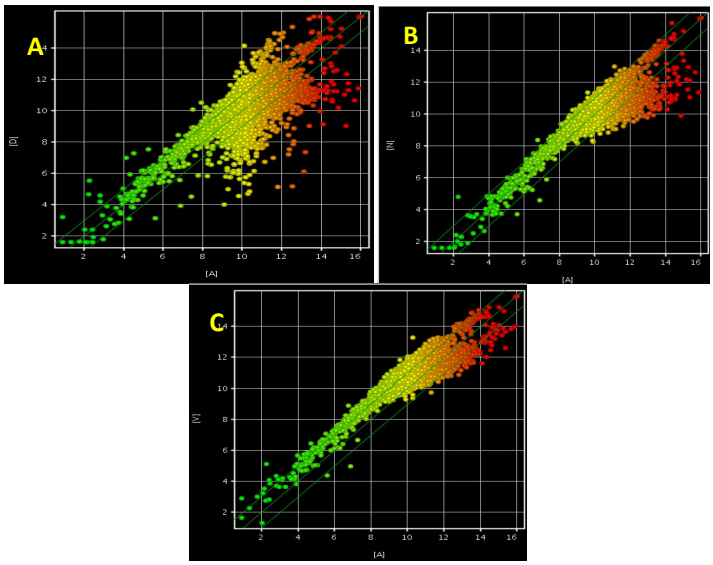
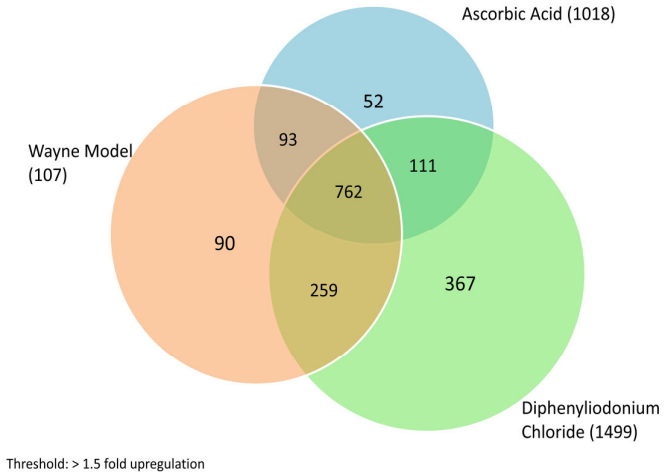


Figure 4.1: Scatter Plot based on Signal Intensities obtained by Genespring software. (A)DPI versus Control, (B) Wayne versus Control and (C) AA versus Control

The number of genes overlapping under three different conditions is represented by Venn diagram (Figure 4.2). 762 genes are up-regulated in all three dormancy models and hence are considered for the next analysis. Like-wise, 1362 genes were down-regulated under three different conditions. This great extent of overlap shows that the DPI treated sample has lot of similarity with known dormancy models. Therefore, DPI treated model can be used as a standard model to study dormancy. Interestingly, 367 genes were found to be exclusively up-regulated and 281 genes were exclusively down-regulated in DPI treated sample (These genes were not detected in other samples because of their very low level of expression which is below the detection level of the experiment) (Figure 4.2)

(A)



(B)

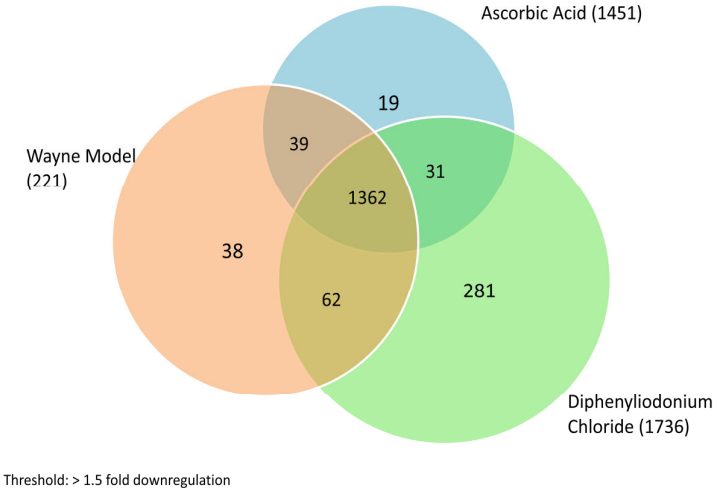


Figure 4.2: Venn diagram of genes differentially expressed under three different conditions**.** (A) Diagrammatic representation of up-regulated genes under 3 different conditions. (B) Diagrammatic representation of down-regulated genes under 3 different conditions.

4.3.1.2 Common genes up-regulated under three different conditions (expression above 1.5 fold)

The relatively large number of differentially expressed genes indicates that the experimental conditions have induced a global shift in gene expression.

Table 4.2: List of genes up-regulated under all three conditions(Expression ratio above1.5fold)

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_5409	MSMEG_5409	hypothetical protein	3.61	3.64	1.83
MSMEG_6882	MSMEG_6882	LysR-family protein transcriptional regulator	2.90	2.49	2.12
MSMEG_6652	MSMEG_6652	hypothetical protein	2.88	3.18	1.65
MSMEG_6854	MSMEG_6854	3-isopropylmalate dehydratase large subunit, EC Number - 4.2.1.33	2.86	2.91	2.06
MSMEG_4139	MSMEG_4139	aliphatic nitrilase	2.82	3.21	1.83
MSMEG_6498	MSMEG_6498	hypothetical protein	2.81	3.19	1.68
MSMEG_0869	MSMEG_0869	hypothetical protein	2.78	3.00	1.75
MSMEG_6654	MSMEG_6654	TnpC protein	2.77	3.23	1.89
MSMEG_0325	MSMEG_0325	hypothetical protein	2.76	2.96	2.56
MSMEG_0273	MSMEG_0273	ethanolamine utilization protein EutN	2.75	2.67	1.74
MSMEG_6123	MSMEG_6123	ABC transporter, membrane spanning protein	2.73	3.12	1.88
MSMEG_1449	MSMEG_1449	integral membrane transporter	2.72	2.91	1.79
MSMEG_0512	MSMEG_0512	BadF,BadG,BcrA,BcrD ATPase family protein	2.65	3.08	1.71
MSMEG_5323	MSMEG_5323	hypothetical protein	2.64	1.83	1.59
MSMEG_6694	MSMEG_6694	hypothetical protein	2.61	2.75	1.84
MSMEG_6534	MSMEG_6534	hypothetical protein	2.61	3.50	1.77
MSMEG_6640	MSMEG_6640	hypothetical protein	2.59	2.51	1.62
MSMEG_6793	MSMEG_6793	hypothetical protein	2.58	2.42	2.42
MSMEG_6495	MSMEG_6495	ABC nitrate,sulfonate,bicarbonate family protein transporter, inner membrane subunit, putative	2.56	2.82	1.54
MSMEG_0514	MSMEG_0514	alpha-galactosidase, EC Number - 3.2.1.22	2.56	2.20	1.71
MSMEG_0635	MSMEG_0635	putative conserved exported protein	2.55	2.28	1.82
MSMEG_1308	MSMEG_1308	peptidase S15	2.55	2.98	1.59
MSMEG_4994	MSMEG_4994	flagella membrane glycoprotein 1B, putative	2.52	2.64	1.74
MSMEG_6878	MSMEG_6878	inner-membrane translocator	2.51	2.15	2.28
MSMEG_0511	MSMEG_0511	putative sugar isomerase, AgaS family protein	2.48	2.64	1.85
MSMEG_0155	MSMEG_0155	transcriptional regulator	2.47	2.40	1.64
MSMEG_0181	MSMEG_0181	alpha-ketoglutarate-dependent taurine dioxygenase, EC Number - 1.14.11.17	2.46	3.10	1.94
MSMEG_0293	MSMEG_0293	Rieske [2Fe-2S] domain protein	2.45	1.77	1.97
MSMEG_5555	MSMEG_5555	antar domain protein	2.44	2.47	2.86
MSMEG_0147	MSMEG_0147	C-5 sterol desaturase, EC Number - 1.3.-.-	2.43	2.66	1.76
MSMEG_3549	ehuD	ectoine,hydroxyectoine ABC transporter, permease protein EhuD	2.42	2.07	1.77
MSMEG_6358	MSMEG_6358	mesocentin	2.42	2.79	2.10
MSMEG_6837	MSMEG_6837	haloalkane dehalogenase, EC Number - 3.8.1.5	2.41	2.54	1.71
MSMEG_0021	panD	aspartate 1-decarboxylase, EC Number - 4.1.1.11	2.41	2.49	2.24
MSMEG_0280	MSMEG_0280	alpha,beta hydrolase fold	2.40	1.93	1.97
MSMEG_6645	MSMEG_6645	2-methylcitrate dehydratase 2, EC Number - 4.2.1.79	2.40	2.49	1.76
MSMEG_6807	MSMEG_6807	transcriptional regulator, TetR family protein	2.39	2.27	1.99
MSMEG_0156	MSMEG_0156	transcriptional regulator, LysR family protein	2.38	16.71	1.94

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_6657	MSMEG_6657	alpha subunit of malonate decarboxylase	2.36	2.23	1.65
MSMEG_0578	MSMEG_0578	acyl-CoA dehydrogenase, middle domain protein	2.36	2.58	1.96
MSMEG_1147	MSMEG_1147	mce related protein	2.34	2.61	1.93
MSMEG_0817	MSMEG_0817	LysR-family protein transcriptional regulator	2.34	2.39	1.71
MSMEG_2154	MSMEG_2154	hypothetical protein	2.34	2.41	1.61
MSMEG_6530	MSMEG_6530	cytochrome c oxidase subunit III family protein	2.33	1.92	2.09
MSMEG_1240	MSMEG_1240	hypothetical protein	2.32	2.22	1.60
MSMEG_6067	rpmG	50S ribosomal protein L33	2.31	2.43	1.91
MSMEG_4782	MSMEG_4782	hypothetical protein	2.31	2.82	1.89
MSMEG_3428	MSMEG_3428	hypothetical protein	2.31	2.66	1.73
MSMEG_6373	MSMEG_6373	hypothetical protein	2.31	2.12	1.84
MSMEG_1175	MSMEG_1175	putative transcriptional regulatory protein	2.30	2.04	1.76
MSMEG_6482	MSMEG_6482	secreted protein	2.28	2.45	1.77
MSMEG_0123	MSMEG_0123	hypothetical protein	2.28	2.54	2.47
MSMEG_0205	MSMEG_0205	tetracenomycin polyketide synthesis hydroxylase TcmH, EC Number - 1.-.-.-	2.28	2.61	1.90
MSMEG_6797	MSMEG_6797	carboxymuconolactone decarboxylase	2.28	2.37	1.94
MSMEG_5411	MSMEG_5411	hypothetical protein	2.28	1.95	2.39
MSMEG_6646	prpB	methylisocitrate lyase, EC Number - 4.1.3.30	2.28	2.19	1.94
MSMEG_6489	MSMEG_6489	hypothetical protein	2.27	2.38	1.71
MSMEG_0299	MSMEG_0299	Rieske [2Fe-2S] domain protein	2.27	2.41	2.01
MSMEG_0481	MSMEG_0481	FAD dependent oxidoreductase	2.27	2.09	1.96
MSMEG_6634	mdcB	triphosphoribosyl-dephospho-CoA synthase MdcB, EC Number - 2.7.8.25	2.26	2.15	1.69
MSMEG_6217	MSMEG_6217	integral membrane protein	2.26	2.56	1.57
MSMEG_6504	MSMEG_6504	hypothetical protein	2.26	2.52	2.22
MSMEG_6296	MSMEG_6296	5-exo-alcohol dehydrogenase, EC Number - 1.1.1.-	2.25	2.14	1.75
MSMEG_6718	MSMEG_6718	transporter, major facilitator family protein	2.25	2.46	1.89
MSMEG_0272	MSMEG_0272	propanediol utilization protein PduA	2.25	2.21	1.80
MSMEG_6833	MSMEG_6833	alcohol dehydrogenase, EC Number - 1.1.1.1	2.24	2.18	2.11
MSMEG_1143	MSMEG_1143	mce related protein	2.24	2.51	1.73
MSMEG_4010	MSMEG_4010	glyoxalase family protein	2.24	2.78	1.62
MSMEG_1086	MSMEG_1086	ABC transporter permease protein	2.24	2.18	1.93
MSMEG_6763	MSMEG_6763	oxidoreductase	2.24	2.38	2.11
MSMEG_3011	MSMEG_3011	steroid monooxygenase	2.24	2.60	1.74
MSMEG_0036	MSMEG_0036	hypothetical protein	2.24	2.19	1.90
MSMEG_1295	MSMEG_1295	transthyretin	2.23	2.14	1.69
MSMEG_5603	MSMEG_5603	transcriptional regulator, ArsR family protein	2.23	2.26	1.77
MSMEG_2998	MSMEG_2998	hypothetical protein	2.22	2.75	1.75
MSMEG_2236	MSMEG_2236	putative thiolase	2.22	2.33	2.31
MSMEG_2875	MSMEG_2875	esterase	2.21	2.51	1.87
MSMEG_6527	MSMEG_6527	transcriptional regulator, TetR family protein	2.21	2.17	1.90
MSMEG_6846	MSMEG_6846	putative C4 decarboxylate transport protein	2.21	2.54	1.85

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_6679	MSMEG_6679	metallo-beta-lactamase family protein	2.21	2.04	1.69
MSMEG_0513	MSMEG_0513	integral membrane protein	2.20	2.36	1.61
MSMEG_0420	MSMEG_0420	hypothetical protein	2.19	2.10	1.94
MSMEG_4444	MSMEG_4444	hypothetical protein	2.19	2.46	2.08
MSMEG_0853	MSMEG_0853	hypothetical protein	2.19	2.20	1.89
MSMEG_6580	MSMEG_6580	transcriptional regulator family protein	2.19	1.98	1.96
MSMEG_5949	MSMEG_5949	hypothetical protein	2.19	2.27	1.60
MSMEG_1224	MSMEG_1224	rifampin ADP-ribosyl transferase	2.18	1.91	1.56
MSMEG_0644	MSMEG_0644	cupin domain protein	2.18	2.25	2.08
MSMEG_0349	MSMEG_0349	virulence factor mce family protein	2.18	2.29	2.17
MSMEG_0276	MSMEG_0276	aldehyde-alcohol dehydrogenase, EC Number - 1.1.1.1 1.2.1.10	2.18	2.27	1.78
MSMEG_6682	MSMEG_6682	RNA polymerase sigma-70 factor, putative	2.17	1.85	1.67
MSMEG_0659	MSMEG_0659	polyamine ABC transporter permease protein	2.17	2.05	1.89
MSMEG_0621	MSMEG_0621	low molecular weight protein antigen 7	2.17	2.09	2.23
MSMEG_0111	MSMEG_0111	putative magnesium transport protein	2.17	2.11	2.43
MSMEG_5971	MSMEG_5971	hypothetical protein	2.16	2.28	2.06
MSMEG_1307	MSMEG_1307	EthD protein	2.16	3.88	1.64
MSMEG_6368	MSMEG_6368	DNA-binding protein	2.16	2.02	2.72
MSMEG_0510	MSMEG_0510	D-tagatose-bisphosphate aldolase, class II, non-catalytic subunit, EC Number - 4.1.2.40	2.15	2.04	1.90
MSMEG_5953	MSMEG_5953	hypothetical protein	2.15	2.04	1.88
MSMEG_6578	MSMEG_6578	hypothetical protein	2.15	2.02	1.75
MSMEG_4069	MSMEG_4069	hypothetical protein	2.14	1.95	2.00
MSMEG_6537	MSMEG_6537	transcriptional regulator, TetR family protein	2.13	2.09	1.59
MSMEG_4173	MSMEG_4173	MaoC family protein	2.13	2.39	1.82
MSMEG_0174	MSMEG_0174	putative inner membrane protein	2.13	2.09	2.02
MSMEG_6560	MSMEG_6560	acyl dehydratase	2.13	1.94	1.62
MSMEG_0707	MSMEG_0707	hypothetical protein	2.12	1.79	1.63
MSMEG_6827	MSMEG_6827	hypothetical protein	2.12	1.96	2.77
MSMEG_1732	MSMEG_1732	Transposase IS116,IS110,IS902 family protein	2.12	2.34	1.54
MSMEG_5819	MSMEG_5819	pyridoxamine 5'-phosphate oxidase family protein	2.12	2.16	1.91
MSMEG_6868	MSMEG_6868	oligopeptide ABC transporter integral membrane protein	2.12	1.96	1.82
MSMEG_6023	MSMEG_6023	transposase	2.12	2.47	1.68
MSMEG_0610	MSMEG_0610	beta-lactamase	2.12	1.98	1.78
MSMEG_6200	MSMEG_6200	hypothetical protein	2.11	1.80	2.07
MSMEG_0320	MSMEG_0320	putative phosphotriesterase	2.11	2.24	1.86
MSMEG_6667	MSMEG_6667	hypothetical protein	2.11	2.00	1.60
MSMEG_1427	MSMEG_1427	transmembrane efflux protein	2.10	2.08	1.81
MSMEG_0518	MSMEG_0518	ABC transporter, nucleotide binding,ATPase protein, sn-Glycerol-3-phosphate	2.10	1.85	2.04
MSMEG_6712	MSMEG_6712	maleylacetate reductase, EC Number - 1.3.1.32	2.10	2.10	1.75
MSMEG_0734	MSMEG_0734	Rieske [2Fe-2S] domain protein	2.10	1.98	1.84
MSMEG_0157	MSMEG_0157	oxalyl-CoA decarboxylase, EC Number - 4.1.1.8	2.10	1.83	2.28

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_6710	MSMEG_6710	hydrolase, alpha,beta fold family protein	2.10	1.85	1.79
MSMEG_6675	MSMEG_6675	hypothetical protein	2.09	2.24	1.79
MSMEG_6510	MSMEG_6510	ABC-type drug export system, membrane protein	2.09	2.00	1.57
MSMEG_3499	MSMEG_3499	hypothetical protein	2.09	1.95	1.89
MSMEG_2531	MSMEG_2531	GntR family protein transcriptional regulator	2.09	1.90	2.52
MSMEG_6572	MSMEG_6572	methyltransferase	2.08	2.03	2.09
MSMEG_1302	MSMEG_1302	alkylphosphonate uptake protein	2.08	2.00	1.59
MSMEG_5955	MSMEG_5955	protein-tyrosine kinase, putative	2.08	2.94	1.78
MSMEG_0407	MSMEG_0407	hypothetical protein	2.08	2.26	2.17
MSMEG_6743	MSMEG_6743	lactoylglutathione lyase	2.08	2.03	1.76
MSMEG_6377	MSMEG_6377	hypothetical protein	2.08	1.87	1.88
MSMEG_5625	MSMEG_5625	cyclododecanone monooxygenase	2.08	1.80	2.90
MSMEG_6334	MSMEG_6334	ABC transporter, quaternary amine uptake transporter QAT family protein, substrate-binding protein	2.08	2.10	1.69
MSMEG_1128	MSMEG_1128	hypothetical protein	2.07	2.01	1.72
MSMEG_1104	MSMEG_1104	hypothetical protein	2.07	2.42	1.99
MSMEG_5035	MSMEG_5035	hypothetical protein	2.07	2.48	1.73
MSMEG_5980	MSMEG_5980	methyltransferase	2.07	2.13	2.06
MSMEG_0660	MSMEG_0660	Bacterial extracellular solute-binding protein	2.07	1.75	1.74
MSMEG_2514	MSMEG_2514	hypothetical protein	2.07	2.60	1.76
MSMEG_6744	MSMEG_6744	oxidoreductase FAD,NAD	2.06	2.11	2.73
MSMEG_0385	MSMEG_0385	hypothetical glycosyl transferase, EC Number - 2.-.-.-	2.06	2.22	2.02
MSMEG_6007	MSMEG_6007	hypothetical protein	2.06	1.92	1.60
MSMEG_5915	MSMEG_5915	enoyl-CoA hydratase	2.06	2.07	1.97
MSMEG_0497	MSMEG_0497	glycerol dehydratase large subunit	2.06	2.08	1.65
MSMEG_0419	MSMEG_0419	integral membrane protein	2.06	2.05	1.65
MSMEG_6442	MSMEG_6442	integral membrane protein	2.06	2.01	1.80
MSMEG_6447	MSMEG_6447	hypothetical protein	2.06	2.01	1.84
MSMEG_6371	MSMEG_6371	GntR-family protein regulator	2.06	2.19	1.87
MSMEG_5022	MSMEG_5022	flavin-containing monooxygenase FMO	2.06	2.21	1.97
MSMEG_0955	MSMEG_0955	hypothetical protein	2.05	1.70	1.86
MSMEG_3938	MSMEG_3938	hypothetical protein	2.05	1.58	1.81
MSMEG_6732	MSMEG_6732	integral membrane protein	2.05	2.25	2.03
MSMEG_6429	MSMEG_6429	hypothetical protein	2.05	1.91	1.73
MSMEG_5340	MSMEG_5340	TetR-family protein transcriptional regulator	2.05	2.20	2.25
MSMEG_0477	MSMEG_0477	hypothetical protein	2.05	1.72	2.05
MSMEG_0852	MSMEG_0852	CBS domain protein	2.05	1.89	1.86
MSMEG_5109	MSMEG_5109	hypothetical protein	2.05	1.88	1.86
MSMEG_1944	MSMEG_1944	membrane protein	2.05	2.25	1.68
MSMEG_2967	MSMEG_2967	hypothetical protein	2.05	2.26	1.83
MSMEG_5930	MSMEG_5930	hypothetical protein	2.04	1.92	2.45
MSMEG_5455	MSMEG_5455	pe family protein	2.04	2.40	1.98

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_1174	MSMEG_1174	cadmium inducible protein cadi	2.04	2.03	1.72
MSMEG_5979	MSMEG_5979	transferase	2.04	1.98	2.27
MSMEG_6231	MSMEG_6231	hypothetical protein	2.04	2.35	1.65
MSMEG_6845	MSMEG_6845	trap dicarboxylate transporter, dctm subunit	2.04	2.19	1.80
MSMEG_6022	MSMEG_6022	xylose repressor, ROK-family protein transcriptional regulator	2.03	1.86	2.07
MSMEG_5627	MSMEG_5627	Rieske [2Fe-2S] domain protein	2.03	1.91	1.84
MSMEG_0259	MSMEG_0259	enoyl-CoA hydratase,isomerase	2.03	2.03	1.85
MSMEG_1137	MSMEG_1137	amino acid permease-associated region	2.03	1.97	1.53
MSMEG_5973	MSMEG_5973	hypothetical protein	2.03	2.16	1.74
MSMEG_6324	MSMEG_6324	peroxidase	2.03	2.19	1.92
MSMEG_1229	gyrB	DNA gyrase, B subunit, EC Number - 5.99.1.3	2.03	2.07	1.73
MSMEG_5905	MSMEG_5905	hypothetical protein	2.03	2.26	1.95
MSMEG_0655	MSMEG_0655	glucose 1-dehydrogenase, putative	2.02	2.11	2.55
MSMEG_4611	MSMEG_4611	carrier protein	2.02	2.04	1.93
MSMEG_0278	MSMEG_0278	hypothetical protein	2.02	1.88	1.75
MSMEG_1457	MSMEG_1457	hypothetical protein	2.02	1.91	1.57
MSMEG_6013	fadD	probable fatty-acid-coa ligase fadd3 fatty-acid-coa synthetase, EC Number - 6.2.1.-	2.02	2.00	2.12
MSMEG_5290	MSMEG_5290	hypothetical protein	2.02	2.18	1.92
MSMEG_5763	MSMEG_5763	hypothetical protein	2.01	2.37	1.91
MSMEG_5717	MSMEG_5717	pyridoxamine 5'-phosphate oxidase family protein	2.01	2.07	1.79
MSMEG_0494	MSMEG_0494	hypothetical protein	2.01	2.13	1.62
MSMEG_5320	MSMEG_5320	hypothetical protein	2.01	2.04	1.94
MSMEG_6717	MSMEG_6717	oxidoreductase, Gfo,Idh,MocA family protein	2.01	2.17	2.07
MSMEG_0297	MSMEG_0297	amidohydrolase	2.01	2.09	1.88
MSMEG_6624	MSMEG_6624	hypothetical protein	2.01	1.85	1.63
MSMEG_1500	MSMEG_1500	TetR family protein transcriptional regulatory protein	2.00	2.06	1.72
MSMEG_6612	moxR	ATPase, MoxR family protein	2.00	1.91	1.60
MSMEG_6890	MSMEG_6890	antibiotic biosynthesis monooxygenase domain protein	2.00	1.70	1.62
MSMEG_0294	fabG	3-oxoacyl-acyl-carrier-protein reductase, EC Number - 1.1.1.100	2.00	2.00	2.05
MSMEG_6475	MSMEG_6475	hypothetical protein	2.00	2.04	1.79
MSMEG_4356	MSMEG_4356	inner membrane ABC transporter permease protein YddQ	2.00	2.36	1.92
MSMEG_4606	MSMEG_4606	hypothetical protein	2.00	2.06	2.00
MSMEG_5998	MSMEG_5998	hypothetical protein	2.00	1.77	1.77
MSMEG_2827	MSMEG_2827	hypothetical protein	1.99	2.07	2.02
MSMEG_6609	MSMEG_6609	hypothetical protein	1.99	1.91	1.88
MSMEG_1374	MSMEG_1374	ribose ABC transporter, periplasmic binding protein	1.99	1.96	1.60
MSMEG_3548	ehuC	ectoine,hydroxyectoine ABC transporter, permease protein EhuC	1.99	2.00	2.23
MSMEG_6333	MSMEG_6333	amino acid ABC transporter, ATP-binding protein	1.99	1.85	1.90
MSMEG_6517	MSMEG_6517	hypothetical protein	1.99	1.89	1.63

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_6658	MSMEG_6658	alpha,beta hydrolase fold	1.99	2.20	2.64
MSMEG_6860	MSMEG_6860	hypothetical protein	1.99	1.98	1.78
MSMEG_0839	MSMEG_0839	ATP-dependent protease La LON domain subfamily protein	1.99	2.06	1.56
MSMEG_6857	MSMEG_6857	putative transcription regulator	1.99	2.03	1.69
MSMEG_1230	MSMEG_1230	hypothetical protein	1.99	1.93	1.76
MSMEG_6347	MSMEG_6347	hypothetical protein	1.99	1.83	1.99
MSMEG_5859	MSMEG_5859	aldehyde dehydrogenase NAD family protein, EC Number - 1.2.1.-	1.99	1.92	1.93
MSMEG_2029	MSMEG_2029	3-ketoacyl-ACP,CoA redutase	1.99	1.96	1.63
MSMEG_6314	MSMEG_6314	haloalkane dehalogenase 1, EC Number - 3.8.1.5	1.98	1.95	2.03
MSMEG_4123	MSMEG_4123	3-hydroxyisobutyrate dehydrogenase	1.98	2.15	1.93
MSMEG_5498	MSMEG_5498	hypothetical protein	1.98	1.91	2.24
MSMEG_2240	MSMEG_2240	cytochrome P450	1.98	2.12	1.87
MSMEG_5147	MSMEG_5147	sugar ABC-transporter integral membrane protein	1.98	1.94	1.84
MSMEG_6812	MSMEG_6812	hypothetical protein	1.98	1.74	1.65
MSMEG_0467	MSMEG_0467	membrane transport protein	1.97	1.91	1.81
MSMEG_0281	MSMEG_0281	choline dehydrogenase	1.97	2.10	1.81
MSMEG_0516	MSMEG_0516	sugar transport system	1.97	1.76	1.67
MSMEG_0442	MSMEG_0442	tetracyclin repressor, C- all-alpha domain protein	1.97	1.82	1.84
MSMEG_5864	MSMEG_5864	cytochrome P450 51	1.97	1.91	1.68
MSMEG_1752	MSMEG_1752	hypothetical protein	1.97	1.87	1.68
MSMEG_2662	MSMEG_2662	hypothetical protein	1.97	1.73	1.96
MSMEG_6604	MSMEG_6604	transcriptional regulator, TetR family protein	1.97	2.24	1.82
MSMEG_4778	MSMEG_4778	putative thiolase	1.97	2.13	2.56
MSMEG_6796	MSMEG_6796	hypothetical protein	1.96	2.15	1.95
MSMEG_6836	MSMEG_6836	putative oxidoreductase	1.96	1.83	1.87
MSMEG_1221	MSMEG_1221	rifampin ADP-ribosyl transferase	1.96	2.20	1.65
MSMEG_0873	MSMEG_0873	hypothetical protein	1.96	1.76	1.69
MSMEG_6556	MSMEG_6556	putative transcriptional regulator	1.96	1.64	1.96
MSMEG_5326	MSMEG_5326	hypothetical protein	1.96	1.82	2.10
MSMEG_3832	MSMEG_3832	4-hydroxyacetophenone monooxygenase	1.96	1.94	1.97
MSMEG_1415	MSMEG_1415	AsnC-family protein transcriptional regulator	1.96	1.90	1.81
MSMEG_6451	MSMEG_6451	transcriptional regulator, ArsR family protein	1.96	2.28	2.12
MSMEG_3339	MSMEG_3339	hypothetical protein	1.96	2.14	1.78
MSMEG_1685	MSMEG_1685	hypothetical protein	1.96	2.24	1.60
MSMEG_5140	MSMEG_5140	nitrate reductase, alpha subunit, EC Number - 1.7.99.4	1.96	2.09	2.40
MSMEG_5356	MSMEG_5356	hypothetical protein	1.95	1.65	2.08
MSMEG_5074	MSMEG_5074	probable transcriptional regulatory protein	1.95	1.99	1.85
MSMEG_6376	MSMEG_6376	transcriptional regulator LacI family protein	1.95	1.90	1.78
MSMEG_6879	MSMEG_6879	integral membrane protein of the ABC-type Nat permease for neutral amino acids NatD	1.95	1.68	1.93
MSMEG_5520	MSMEG_5520	hypothetical protein	1.95	1.74	2.04
MSMEG_1625	MSMEG_1625	amino acid transporter, putative	1.95	1.80	1.58

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_0486	MSMEG_0486	putative ABC transporter, periplasmic protein	1.95	1.92	1.84
MSMEG_0271	MSMEG_0271	hypothetical protein	1.95	1.87	2.06
MSMEG_5997	MSMEG_5997	hypothetical protein	1.94	2.42	1.71
MSMEG_0189	MSMEG_0189	anhydro-N-acetylmuramic acid kinase	1.94	1.79	1.86
MSMEG_4840	MSMEG_4840	Rieske [2Fe-2S] domain protein	1.94	1.98	1.77
MSMEG_1789	MSMEG_1789	hypothetical protein	1.94	1.78	1.55
MSMEG_6460	MSMEG_6460	hypothetical protein	1.94	2.09	1.97
MSMEG_6304	MSMEG_6304	ATP-dependent DNA ligase	1.94	2.12	1.87
MSMEG_0496	MSMEG_0496	coenzyme B12-dependent glycerol dehydrogenase small subunit	1.94	2.09	1.88
MSMEG_6691	MSMEG_6691	glutamine amidotransferase	1.94	2.04	2.01
MSMEG_5932	MSMEG_5932	hypothetical protein	1.94	1.80	2.01
MSMEG_6731	MSMEG_6731	transcriptional regulatory protein	1.94	1.94	1.80
MSMEG_0279	MSMEG_0279	amino acid permease	1.94	1.61	1.87
MSMEG_0595	MSMEG_0595	glycolate oxidase	1.94	2.10	1.78
MSMEG_0664	MSMEG_0664	FAD dependent oxidoreductase	1.94	2.02	1.75
MSMEG_2221	MSMEG_2221	hypothetical protein	1.94	1.87	1.67
MSMEG_0526	MSMEG_0526	oxidoreductase, GMC family protein	1.93	1.89	1.69
MSMEG_6886	MSMEG_6886	transcriptional regulator	1.93	2.13	2.21
MSMEG_2185	MSMEG_2185	hypothetical protein	1.93	2.06	2.02
MSMEG_6823	MSMEG_6823	short-chain dehydrogenase,reductase SDR	1.93	1.58	2.01
MSMEG_1984	MSMEG_1984	haloacetate dehalogenase H-1, EC Number - 3.8.1.3	1.93	2.18	1.56
MSMEG_1429	MSMEG_1429	cytochrome P450-terp, EC Number - 1.14.-.-	1.93	1.70	1.79
MSMEG_0215	MSMEG_0215	YhhW family protein	1.93	2.13	1.83
MSMEG_6121	MSMEG_6121	dipeptide transport ATP-binding protein DppD	1.93	1.88	1.61
MSMEG_6018	MSMEG_6018	xylose transport system permease protein XylH, EC Number - 3.6.3.17	1.93	1.83	1.78
MSMEG_0462	MSMEG_0462	MmpS4 protein	1.93	2.07	1.81
MSMEG_6476	MSMEG_6476	hypothetical protein	1.93	2.13	1.85
MSMEG_3420	MSMEG_3420	gluconate 5-dehydrogenase, EC Number - 1.1.1.69	1.93	2.01	1.95
MSMEG_6813	MSMEG_6813	amidohydrolase 2	1.92	2.17	1.99
MSMEG_1232	MSMEG_1232	ABC transporter substrate-binding protein	1.92	1.92	1.57
MSMEG_0292	MSMEG_0292	hypothetical protein	1.92	1.68	1.93
MSMEG_0182	MSMEG_0182	epoxide hydrolase 1, EC Number - 3.3.2.3	1.92	2.07	1.91
MSMEG_6666	MSMEG_6666	hypothetical protein	1.92	1.87	1.79
MSMEG_6260	glnT	glutamine synthetase, type III, EC Number - 6.3.1.2	1.92	1.92	1.77
MSMEG_2904	MSMEG_2904	MmcI protein	1.92	2.40	1.91
MSMEG_0128	MSMEG_0128	thioesterase superfamily protein	1.92	1.99	2.10
MSMEG_0305	MSMEG_0305	acyltransferase domain protein	1.91	2.25	1.94
MSMEG_0151	MSMEG_0151	PntAB protein	1.91	2.07	1.60
MSMEG_1228	MSMEG_1228	sulfatase	1.91	1.85	1.70
MSMEG_5357	MSMEG_5357	hypothetical protein	1.91	1.78	1.60
MSMEG_2882	MSMEG_2882	5-exo-alcohol dehydrogenase, EC Number -	1.91	2.06	2.05

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
		1.1.1.-			
MSMEG_1591	MSMEG_1591	TnpC protein, similar to the proteins: MSMEG_1310	1.91	2.11	2.42
MSMEG_0706	MSMEG_0706	4-carboxy-4-hydroxy-2-oxoadipate aldolase	1.91	1.85	1.85
MSMEG_5504	MSMEG_5504	conserved secreted protein	1.91	1.52	1.96
MSMEG_5960	MSMEG_5960	O-antigen polymerase, putative	1.91	1.68	1.75
MSMEG_0122	MSMEG_0122	putative periplasmic solute-binding protein	1.91	1.87	1.79
MSMEG_6233	MSMEG_6233	hypothetical protein	1.91	1.85	1.58
MSMEG_4409	MSMEG_4409	hypothetical protein	1.90	2.25	1.85
MSMEG_1304	MSMEG_1304	rhizopine catabolism protein	1.90	1.88	1.85
MSMEG_2524	MSMEG_2524	ABC transporter, ATP-binding protein	1.90	1.95	1.76
MSMEG_5300	MSMEG_5300	short-chain type dehydrogenase,reductase, EC Number - 1.-.-.-	1.90	1.99	2.21
MSMEG_1974	MSMEG_1974	propane monooxygenase coupling protein	1.90	1.88	1.77
MSMEG_6492	MSMEG_6492	GAF domain protein, putative	1.90	1.97	1.87
MSMEG_0982	MSMEG_0982	immunogenic protein	1.90	1.78	1.81
MSMEG_0161	MSMEG_0161	formate dehydrogenase, alpha subunit, EC Number - 1.2.1.2	1.90	1.85	1.81
MSMEG_3311	MSMEG_3311	acyl carrier protein	1.90	2.26	1.87
MSMEG_6803	MSMEG_6803	ribose transport system permease protein RbsC, EC Number - 3.6.3.17	1.90	1.89	1.64
MSMEG_6834	MSMEG_6834	alcohol dehydrogenase	1.90	1.96	2.09
MSMEG_0332	MSMEG_0332	2-nitropropane dioxygenase, NPD	1.90	2.05	1.87
MSMEG_4459	speB	agmatinase, EC Number - 3.5.3.11	1.90	2.15	2.06
MSMEG_4802	MSMEG_4802	hypothetical protein	1.90	1.90	2.33
MSMEG_0039	MSMEG_0039	small membrane hydrophobic protein	1.90	1.87	1.92
MSMEG_4158	MSMEG_4158	putative transposase	1.90	1.67	1.79
MSMEG_6500	MSMEG_6500	hypothetical protein	1.89	1.82	1.81
MSMEG_6546	MSMEG_6546	transcriptional regulatory protein	1.89	1.82	1.57
MSMEG_5586	MSMEG_5586	hypothetical protein	1.89	2.49	1.89
MSMEG_5316	MSMEG_5316	glutamine ABC transporter, ATP-binding protein	1.89	2.13	2.17
MSMEG_1333	MSMEG_1333	hypothetical protein	1.89	2.03	1.66
MSMEG_5629	MSMEG_5629	hypothetical protein	1.89	1.97	2.00
MSMEG_1792	MSMEG_1792	hypothetical protein	1.89	1.96	2.24
MSMEG_6866	MSMEG_6866	dipeptide transport system permease protein DppB	1.89	2.15	1.69
MSMEG_5573	MSMEG_5573	sugar ABC transporter permease protein	1.89	2.06	1.71
MSMEG_5990	MSMEG_5990	lipid-transfer protein	1.89	1.82	2.65
MSMEG_5856	pcaC	4-carboxymuconolactone decarboxylase domain protein, EC Number - 4.1.1.44	1.88	2.02	1.65
MSMEG_6819	MSMEG_6819	dehydrogenase	1.88	1.87	2.00
MSMEG_1975	MSMEG_1975	amidohydrolase 2	1.88	1.95	2.13
MSMEG_0657	MSMEG_0657	Rieske 2Fe-2S domain protein	1.88	1.91	1.78
MSMEG_1088	gatA	glutamyl-tRNAGln,aspartyl-tRNAAsn amidotransferase, A subunit, EC Number - 6.3.5.-	1.88	1.92	1.62
MSMEG_6835	MSMEG_6835	Fatty acid desaturase	1.88	1.74	2.61
MSMEG_0478	MSMEG_0478	secreted protein, putative	1.88	1.81	2.19

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_2257	MSMEG_2257	cytochrome P450-terp, EC Number - 1.14.-.-	1.88	1.83	1.84
MSMEG_0723	MSMEG_0723	hypothetical protein	1.88	1.68	2.00
MSMEG_0900	MSMEG_0900	eptc-inducible aldehyde dehydrogenase, EC Number - 1.2.1.3	1.88	1.89	2.05
MSMEG_0357	MSMEG_0357	transmembrane transport protein	1.88	1.70	1.87
MSMEG_4045	MSMEG_4045	muconolactone delta-isomerase, EC Number - 5.3.3.4	1.88	1.62	1.85
MSMEG_0329	MSMEG_0329	FMN oxidoreductase	1.88	1.92	1.73
MSMEG_1587	MSMEG_1587	alpha,beta hydrolase fold	1.88	2.08	1.99
MSMEG_6426	MSMEG_6426	hypothetical protein	1.88	1.71	1.78
MSMEG_0355	MSMEG_0355	hypothetical protein	1.88	1.68	2.55
MSMEG_5991	MSMEG_5991	MaoC like domain, putative	1.88	1.82	2.20
MSMEG_0519	MSMEG_0519	hypothetical protein	1.87	1.65	2.30
MSMEG_5335	MSMEG_5335	formamidase, EC Number - 3.5.1.49	1.87	1.73	2.10
MSMEG_2129	MSMEG_2129	hypothetical protein	1.87	1.95	2.19
MSMEG_0653	MSMEG_0653	hypothetical protein	1.87	2.15	2.16
MSMEG_5418	MSMEG_5418	iron permease FTR1	1.87	2.16	1.77
MSMEG_6701	MSMEG_6701	amino acid permease	1.87	2.09	1.88
MSMEG_4787	MSMEG_4787	virulence factor mce family protein	1.87	1.92	2.26
MSMEG_5034	MSMEG_5034	hypothetical protein	1.87	2.24	1.83
MSMEG_6224	MSMEG_6224	Retinal pigment epithelial membrane protein	1.87	1.82	2.03
MSMEG_0441	MSMEG_0441	hypothetical protein	1.87	1.88	2.10
MSMEG_6147	MSMEG_6147	hypothetical protein	1.87	1.54	2.07
MSMEG_0112	MSMEG_0112	hypothetical protein	1.87	1.71	1.76
MSMEG_0799	MSMEG_0799	hypothetical protein	1.87	2.09	1.65
MSMEG_5945	MSMEG_5945	hypothetical protein	1.87	1.70	2.27
MSMEG_0480	MSMEG_0480	transcriptional regulator, GntR family protein	1.87	2.08	2.78
MSMEG_5348	MSMEG_5348	medium-chain fatty acid-CoA ligase	1.87	2.02	2.05
MSMEG_6791	MSMEG_6791	3-hydroxybutyryl-CoA dehydrogenase	1.87	1.88	2.08
MSMEG_1216	MSMEG_1216	ABC-type transport system periplasmic substrate-binding protein	1.87	1.85	1.67
MSMEG_6794	MSMEG_6794	DNA-binding protein	1.87	1.85	2.16
MSMEG_6558	MSMEG_6558	putative enoyl-CoA hydratase	1.87	2.31	1.86
MSMEG_0785	MSMEG_0785	hypothetical protein	1.87	1.95	1.95
MSMEG_1454	MSMEG_1454	hypothetical protein	1.87	1.97	1.90
MSMEG_1972	MSMEG_1972	methane monooxygenase component C, EC Number - 1.14.13.25	1.86	1.96	1.72
MSMEG_0620	MSMEG_0620	pe family protein	1.86	1.78	1.61
MSMEG_1785	MSMEG_1785	hypothetical protein	1.86	1.94	1.59
MSMEG_0508	MSMEG_0508	glycerol-phosphate porter	1.86	1.90	2.05
MSMEG_0330	MSMEG_0330	transcriptional regulator, LuxR family protein	1.86	1.74	2.10
MSMEG_6273	MSMEG_6273	integral membrane protein	1.86	1.74	1.66
MSMEG_6681	MSMEG_6681	hypothetical protein	1.86	1.68	2.08
MSMEG_3393	MSMEG_3393	putative acyl-CoA dehydrogenase	1.86	1.94	1.91
MSMEG_6635	MSMEG_6635	alpha subunit of malonate decarboxylase	1.86	1.84	1.57

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_6325	MSMEG_6325	hypothetical protein	1.85	1.83	2.32
MSMEG_6305	MSMEG_6305	hypothetical protein	1.85	1.83	1.91
MSMEG_0475	MSMEG_0475	nucleotide-sugar dehydrogenase	1.85	1.91	1.99
MSMEG_6633	mdcC	malonate decarboxylase acyl carrier protein	1.85	2.17	1.82
MSMEG_6715	MSMEG_6715	AP endonuclease, family protein 2	1.85	1.84	2.04
MSMEG_2856	MSMEG_2856	virulence factor Mce family protein	1.85	2.14	1.90
MSMEG_0996	MSMEG_0996	hypothetical protein	1.85	2.13	1.90
MSMEG_5374	MSMEG_5374	glutamate--ammonia ligase	1.85	1.64	1.59
MSMEG_1797	MSMEG_1797	salicylate esterase	1.85	2.07	1.78
MSMEG_1458	MSMEG_1458	tena,thi-4 family protein	1.85	1.95	1.70
MSMEG_4425	MSMEG_4425	putative oxidoreductase	1.85	1.99	1.99
MSMEG_5982	MSMEG_5982	UDP-glucose 6-dehydrogenase, EC Number - 1.1.1.22	1.85	1.95	2.48
MSMEG_6375	MSMEG_6375	transporter, major facilitator family protein	1.85	1.93	1.80
MSMEG_0356	MSMEG_0356	acetyltransferase	1.85	1.52	2.18
MSMEG_1614	MSMEG_1614	glutamine transport ATP-binding protein GlnQ	1.84	3.30	1.70
MSMEG_5834	MSMEG_5834	metallo-beta-lactamase superfamily protein	1.84	1.96	2.38
MSMEG_0527	MSMEG_0527	2-hydroxycyclohexnecarboxyl-CoA dehydrogenase	1.84	1.73	1.69
MSMEG_6490	MSMEG_6490	major facilitator superfamily protein	1.84	2.11	1.65
MSMEG_4605	MSMEG_4605	hypothetical protein	1.84	2.07	1.96
MSMEG_4824	MSMEG_4824	hypothetical protein	1.84	1.79	2.13
MSMEG_0875	MSMEG_0875	putative sialic acid transporter	1.84	1.79	1.60
MSMEG_6826	MSMEG_6826	L-lactate permease	1.84	2.08	1.70
MSMEG_6550	MSMEG_6550	hypothetical protein	1.84	1.92	1.59
MSMEG_1504	MSMEG_1504	probable antibiotic-transport integral membrane leucine and alanine and valine rich protein abc transporter	1.84	1.89	1.75
MSMEG_0745	MSMEG_0745	[2Fe-2S] binding domain protein	1.84	1.81	1.71
MSMEG_6650	MSMEG_6650	hypothetical protein	1.84	1.61	1.81
MSMEG_6883	MSMEG_6883	LysR-family protein transcriptional regulator	1.84	1.63	1.84
MSMEG_1566	MSMEG_1566	oxidoreductase	1.84	1.98	2.31
MSMEG_6840	MSMEG_6840	LysR-family protein transcriptional regulator	1.84	1.90	1.77
MSMEG_2232	MSMEG_2232	amidohydrolase family protein	1.84	1.73	1.80
MSMEG_2234	MSMEG_2234	acyl-CoA dehydrogenase, middle domain protein	1.84	1.90	1.68
MSMEG_1709	MSMEG_1709	inner membrane ABC transporter permease protein YjfF	1.84	1.78	1.56
MSMEG_5364	MSMEG_5364	amidohydrolase 2	1.83	1.70	2.24
MSMEG_6607	MSMEG_6607	hypothetical protein	1.83	1.86	1.68
MSMEG_6805	MSMEG_6805	beta-lactamase	1.83	1.82	1.84
MSMEG_6068	rpmB	ribosomal protein L28	1.83	1.76	2.12
MSMEG_0872	MSMEG_0872	twin-arginine translocation pathway signal	1.83	1.59	1.68
MSMEG_2230	MSMEG_2230	amidohydrolase family protein	1.83	1.68	1.97
MSMEG_2011	MSMEG_2011	transcriptional regulator, LacI family protein	1.83	1.97	1.70
MSMEG_5950	MSMEG_5950	putative colanic acid biosynthesis acetyltransferase WcaF, EC Number - 2.3.1.-	1.83	1.61	2.76

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_5838	MSMEG_5838	TetR-family protein transcriptional regulator	1.83	1.58	2.46
MSMEG_6242	MSMEG_6242	alcohol dehydrogenase, iron-containing, EC Number - 1.1.1.1	1.83	1.63	2.34
MSMEG_1197	MSMEG_1197	transcriptional regulator, LuxR family protein	1.83	1.75	1.79
MSMEG_5313	MSMEG_5313	hypothetical protein	1.83	1.94	1.86
MSMEG_0348	MSMEG_0348	mce-family protein mce3c	1.83	1.77	1.86
MSMEG_0523	MSMEG_0523	DNA-binding protein	1.82	1.90	1.83
MSMEG_4438	MSMEG_4438	hypothetical protein	1.82	1.85	1.85
MSMEG_3334	MSMEG_3334	hypothetical protein	1.82	2.14	1.78
MSMEG_6790	MSMEG_6790	AP endonuclease, family protein 2	1.82	1.77	1.85
MSMEG_6820	surE	acid phosphatase SurE, EC Number - 3.1.3.2	1.82	1.89	1.72
MSMEG_6122	MSMEG_6122	ABC transporter, membrane spanning protein	1.82	1.67	2.24
MSMEG_0260	MSMEG_0260	DNA-binding protein	1.82	1.84	1.88
MSMEG_4006	MSMEG_4006	transcriptional regulator, CdaR, putative	1.82	2.00	2.15
MSMEG_1300	MSMEG_1300	hypothetical protein	1.82	2.19	1.99
MSMEG_2254	MSMEG_2254	oxalate decarboxylase OxdC, putative, EC Number - 4.1.1.2	1.82	2.12	1.57
MSMEG_0284	MSMEG_0284	ribosyldihydronicotinamide dehydrogenase [quinone], EC Number - 1.10.99.2	1.82	1.66	1.59
MSMEG_3095	MSMEG_3095	D-ribose-binding periplasmic protein, EC Number - 3.6.3.17	1.81	1.81	1.74
MSMEG_3133	MSMEG_3133	hypothetical protein	1.81	2.17	1.68
MSMEG_4416	MSMEG_4416	hypothetical protein	1.81	2.11	1.73
MSMEG_5738	MSMEG_5738	hypothetical protein	1.81	2.00	2.17
MSMEG_1384	MSMEG_1384	hypothetical protein	1.81	1.68	1.92
MSMEG_1626	MSMEG_1626	putative DNA-binding protein	1.81	1.84	1.56
MSMEG_0661	MSMEG_0661	glutamate-1-semialdehyde 2,1-aminomutase, EC Number - 5.4.3.8	1.81	1.86	1.78
MSMEG_0803	MSMEG_0803	hypothetical protein	1.81	1.91	1.65
MSMEG_5476	gcl	glyoxylate carboligase, EC Number - 4.1.1.47	1.81	1.83	1.84
MSMEG_0789	thiE	thiamine-phosphate pyrophosphorylase, EC Number - 2.5.1.3	1.81	1.90	1.68
MSMEG_6496	MSMEG_6496	nitrate,sulfonate,bicarbonate ABC transporter ATPase subunit	1.81	1.87	1.68
MSMEG_1973	MSMEG_1973	methane monooxygenase component C	1.81	2.19	1.90
MSMEG_5910	MSMEG_5910	quinone binding protein	1.81	1.77	2.40
MSMEG_4884	MSMEG_4884	hypothetical protein	1.81	1.72	2.13
MSMEG_0257	MSMEG_0257	acyl-CoA synthetase	1.81	1.78	1.88
MSMEG_2229	MSMEG_2229	enoyl-CoA hydratase	1.81	2.04	1.71
MSMEG_0443	MSMEG_0443	hydrolase, carbon-nitrogen family protein	1.81	1.94	1.80
MSMEG_0333	MSMEG_0333	Carboxyl transferase domain protein	1.81	1.90	1.82
MSMEG_0390	MSMEG_0390	putative acyltransferase	1.80	1.77	1.88
MSMEG_4780	MSMEG_4780	cytochrome p450	1.80	1.88	1.81
MSMEG_6714	MSMEG_6714	hypothetical protein	1.80	1.88	1.68
MSMEG_0772	MSMEG_0772	phytase	1.80	1.91	1.84
MSMEG_1543	MSMEG_1543	eptc-inducible aldehyde dehydrogenase, EC Number - 1.2.1.3	1.80	1.90	1.93
MSMEG_3553	hydA	dihydropyrimidinase, EC Number - 3.5.2.2	1.80	1.84	2.03

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_5502	MSMEG_5502	hypothetical protein	1.80	1.84	1.88
MSMEG_5995	MSMEG_5995	P450 heme-thiolate protein	1.80	1.80	1.93
MSMEG_5397	recQ	ATP-dependent DNA helicase RecQ, EC Number - 3.6.1.-	1.80	1.93	1.96
MSMEG_1705	MSMEG_1705	D-xylose transport ATP-binding protein XylG, EC Number - 3.6.3.17	1.80	1.66	1.64
MSMEG_0515	MSMEG_0515	probable sugar transporter sugar binding lipoprotein	1.79	1.68	2.24
MSMEG_2877	MSMEG_2877	hypothetical protein	1.79	1.86	1.79
MSMEG_0054	MSMEG_0054	ISMsm2, transposase, similar to the proteins: MSMEG_3510 ; MSMEG_4522 ; MSMEG_5669	1.79	2.00	1.78
MSMEG_5339	MSMEG_5339	nitrile hydratase regulator1	1.79	1.89	2.01
MSMEG_5921	MSMEG_5921	hypothetical protein	1.79	1.77	1.92
MSMEG_5944	MSMEG_5944	glucose-methanol-choline oxidoreductase	1.79	1.78	1.76
MSMEG_6118	MSMEG_6118	IclR-family protein transcriptional regulator	1.79	1.75	1.83
MSMEG_0150	MSMEG_0150	NADP transhydrogenase beta subunit	1.79	1.91	1.92
MSMEG_3274	MSMEG_3274	MerR-family protein transcriptional regulator	1.79	1.77	3.61
MSMEG_5546	MSMEG_5546	membrane spanning protein	1.79	1.78	1.64
MSMEG_3293	MSMEG_3293	hypothetical protein	1.79	1.76	2.28
MSMEG_1794	MSMEG_1794	dehydrogenase	1.79	1.88	1.60
MSMEG_5457	MSMEG_5457	shikimate 5-dehydrogenase	1.79	1.86	1.88
MSMEG_2253	MSMEG_2253	hypothetical protein	1.79	2.85	1.90
MSMEG_0146	MSMEG_0146	mosc domain protein	1.78	1.74	1.76
MSMEG_6768	MSMEG_6768	halogenase	1.78	1.64	1.87
MSMEG_2532	aroQ	dehydroquinase class II, EC Number - 4.2.1.10	1.78	1.92	1.69
MSMEG_0426	MSMEG_0426	transcriptional regulator, GntR family protein	1.78	1.87	1.69
MSMEG_5815	MSMEG_5815	betaine aldehyde dehydrogenase, EC Number - 1.2.1.8	1.78	1.87	1.78
MSMEG_4035	MSMEG_4035	citrate synthase	1.78	1.80	2.03
MSMEG_0339	MSMEG_0339	FMN-dependent monooxygenase	1.78	1.69	1.84
MSMEG_1324	MSMEG_1324	hypothetical protein	1.78	1.94	1.79
MSMEG_0632	MSMEG_0632	hypothetical protein	1.78	1.61	1.70
MSMEG_5494	MSMEG_5494	acyl-CoA dehydrogenase fadE12, EC Number - 1.3.99.-	1.78	1.86	1.78
MSMEG_1581	MSMEG_1581	hypothetical protein	1.78	1.55	1.65
MSMEG_5506	MSMEG_5506	hypothetical protein	1.78	1.85	2.41
MSMEG_5499	MSMEG_5499	hypothetical protein	1.78	1.53	1.90
MSMEG_1223	MSMEG_1223	hypothetical protein	1.78	1.78	1.71
MSMEG_4835	MSMEG_4835	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	1.78	1.68	1.89
MSMEG_1227	MSMEG_1227	transcriptional regulator, GntR family protein	1.78	1.82	2.07
MSMEG_1696	MSMEG_1696	regulatory protein, MarR	1.78	2.12	1.67
MSMEG_6828	MSMEG_6828	transcriptional regulator	1.78	1.96	2.36
MSMEG_1406	MSMEG_1406	hypothetical protein	1.78	1.84	1.73
MSMEG_0137	MSMEG_0137	virulence factor mce family protein	1.78	1.80	1.90
MSMEG_6871	MSMEG_6871	isomerase	1.77	1.78	1.87
MSMEG_5604	MSMEG_5604	integral membrane protein	1.77	2.17	2.51

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_6811	MSMEG_6811	hypothetical protein	1.77	1.84	2.08
MSMEG_6713	MSMEG_6713	hydroxyquinol 1,2-dioxygenase, EC Number - 1.13.11.37	1.77	1.88	1.91
MSMEG_4384	MSMEG_4384	Penicillin binding protein transpeptidase domain protein	1.77	2.34	1.86
MSMEG_4169	MSMEG_4169	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	1.77	2.04	1.93
MSMEG_6792	MSMEG_6792	inner membrane permease YgbN	1.77	1.70	1.69
MSMEG_1488	MSMEG_1488	acetolactate synthase	1.77	2.18	1.82
MSMEG_5931	MSMEG_5931	short chain dehydrogenase	1.77	1.57	2.00
MSMEG_6551	MSMEG_6551	hypothetical protein	1.77	1.86	1.91
MSMEG_4417	msrA	methionine-S-sulfoxide reductase, EC Number - 1.8.4.6	1.77	2.04	2.03
MSMEG_4156	MSMEG_4156	putative transposase	1.77	2.09	1.65
MSMEG_5600	MSMEG_5600	hypothetical protein	1.77	1.89	2.12
MSMEG_1908	MSMEG_1908	benzoate 1,2-dioxygenase alpha subunit, EC Number - 1.14.12.10	1.77	2.39	1.98
MSMEG_6553	MSMEG_6553	ABC transporter ATP-binding protein	1.77	1.85	1.75
MSMEG_1156	MSMEG_1156	dihydrodipicolinate synthetase	1.77	1.85	1.84
MSMEG_2539	MSMEG_2539	thiopurine S-methyltransferase tpmt superfamily protein	1.77	1.82	1.87
MSMEG_0605	MSMEG_0605	hypothetical protein	1.77	1.83	2.18
MSMEG_4822	MSMEG_4822	hypothetical protein	1.76	1.97	2.61
MSMEG_4963	MSMEG_4963	peptide synthetase ScpsB, putative	1.76	1.87	2.15
MSMEG_0298	MSMEG_0298	hypothetical protein	1.76	1.78	1.95
MSMEG_5978	MSMEG_5978	GlcNAc-PI de-N-acetylase family protein	1.76	1.62	1.87
MSMEG_5919	MSMEG_5919	hypothetical protein	1.76	1.58	1.82
MSMEG_0149	MSMEG_0149	thiamine biosynthesis protein ThiC	1.76	1.81	2.13
MSMEG_2570	MSMEG_2570	xanthine,uracil permease	1.76	1.56	1.95
MSMEG_0163	MSMEG_0163	hypothetical protein	1.76	1.83	2.25
MSMEG_0164	MSMEG_0164	transmembrane transport protein	1.76	1.87	2.75
MSMEG_6117	MSMEG_6117	glucarate dehydratase, EC Number - 4.2.1.40	1.76	1.93	1.72
MSMEG_6449	MSMEG_6449	hypothetical protein	1.76	1.61	1.72
MSMEG_4988	MSMEG_4988	integral membrane protein	1.76	1.82	2.19
MSMEG_6887	MSMEG_6887	amidohydrolase family protein	1.76	1.75	1.99
MSMEG_2243	MSMEG_2243	isochorismatase family protein	1.76	2.27	1.67
MSMEG_1414	MSMEG_1414	Amidinotransferase	1.75	1.75	2.24
MSMEG_6573	MSMEG_6573	hypothetical protein	1.75	1.95	1.80
MSMEG_6390	MSMEG_6390	transporter, major facilitator family protein	1.75	2.03	1.96
MSMEG_0346	MSMEG_0346	virulence factor	1.75	1.74	1.94
MSMEG_1198	MSMEG_1198	hypothetical protein	1.75	1.78	1.82
MSMEG_1148	MSMEG_1148	mce related protein	1.75	1.82	1.64
MSMEG_3411	MSMEG_3411	MOSC domain protein	1.75	1.96	1.78
MSMEG_3439	MSMEG_3439	hypothetical protein	1.75	1.86	1.89
MSMEG_0427	nirB	nitrite reductase [NADPH], large subunit, EC Number - 1.7.1.4	1.75	1.74	1.88
MSMEG_1989	MSMEG_1989	phenoxybenzoate dioxygenase beta subunit, EC Number - 1.-.-.-	1.75	2.02	2.00

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_3817	MSMEG_3817	hypothetical protein	1.75	1.76	1.99
MSMEG_6769	MSMEG_6769	transporter, monovalent cation:proton antiporter-2 CPA2 family protein	1.75	2.01	1.83
MSMEG_5376	MSMEG_5376	hypothetical protein	1.75	1.94	1.62
MSMEG_5654	MSMEG_5654	transcriptional regulator, LysR family protein	1.75	2.36	2.41
MSMEG_1844	MSMEG_1844	hypothetical protein	1.75	1.63	1.57
MSMEG_5958	MSMEG_5958	hypothetical protein	1.75	1.97	1.67
MSMEG_3104	MSMEG_3104	hypothetical protein	1.75	1.88	1.80
MSMEG_5972	MSMEG_5972	hypothetical protein	1.75	1.70	2.19
MSMEG_5387	MSMEG_5387	hypothetical protein	1.74	1.70	1.87
MSMEG_5299	MSMEG_5299	hypothetical protein	1.74	1.92	1.71
MSMEG_1386	MSMEG_1386	hypothetical protein	1.74	1.84	2.05
MSMEG_4433	MSMEG_4433	dehydrogenase	1.74	2.13	2.06
MSMEG_6243	MSMEG_6243	response regulator receiver domain protein	1.74	2.04	1.75
MSMEG_6588	MSMEG_6588	fumarylacetoacetate	1.74	2.16	2.05
MSMEG_1226	MSMEG_1226	sulfatase-modifying factor 1	1.74	1.82	1.61
MSMEG_5385	MSMEG_5385	metallo-beta-lactamase family protein	1.74	2.05	2.58
MSMEG_0326	MSMEG_0326	AMP-dependent synthetase and ligase	1.74	1.73	1.97
MSMEG_0466	MSMEG_0466	hypothetical protein	1.74	1.65	1.87
MSMEG_0418	MSMEG_0418	succinate dehydrogenase flavoprotein subunit, EC Number - 1.3.99.1	1.74	1.68	1.81
MSMEG_0517	MSMEG_0517	sugar binding-protein dependent transporter system permease	1.73	1.86	1.68
MSMEG_5456	MSMEG_5456	MK35 lipoprotein	1.73	1.83	1.92
MSMEG_3986	MSMEG_3986	acetyl-coenzyme A synthetase, EC Number - 6.2.1.1	1.73	1.77	1.77
MSMEG_5564	MSMEG_5564	NADPH dehydrogenase, quinone family protein	1.73	1.69	1.99
MSMEG_0742	MSMEG_0742	probable transcriptional regulatory protein	1.73	1.65	1.91
MSMEG_2149	MSMEG_2149	hypothetical protein	1.73	1.80	1.66
MSMEG_6538	MSMEG_6538	3-oxoacyl-acyl-carrier-protein reductase, putative	1.73	1.63	1.58
MSMEG_4316	MSMEG_4316	methylated-DNA--protein-cysteine methyltransferase, EC Number - 2.1.1.63	1.73	1.86	2.04
MSMEG_6505	MSMEG_6505	NfnB protein	1.73	1.64	1.98
MSMEG_1699	MSMEG_1699	pentachlorophenol 4-monooxygenase	1.73	2.18	1.62
MSMEG_2549	MSMEG_2549	major facilitator superfamily protein	1.73	1.64	2.07
MSMEG_5289	MSMEG_5289	hypothetical protein	1.73	2.23	1.72
MSMEG_6348	MSMEG_6348	hypothetical protein	1.73	1.59	1.81
MSMEG_6615	MSMEG_6615	hypothetical protein	1.73	1.82	2.82
MSMEG_6323	MSMEG_6323	hypothetical protein	1.73	1.88	1.99
MSMEG_2968	MSMEG_2968	hypothetical protein	1.73	2.18	1.85
MSMEG_2068	MSMEG_2068	hypothetical protein	1.72	1.85	1.77
MSMEG_6660	MSMEG_6660	permease, cytosine,purines, uracil, thiamine, allantoin family protein	1.72	1.82	1.66
MSMEG_6931	MSMEG_6931	RNA polymerase sigma-70 factor	1.72	1.88	1.93
MSMEG_1719	MSMEG_1719	ISMsm8, transposase	1.72	1.69	1.71
MSMEG_4424	MSMEG_4424	endoribonuclease L-PSP	1.72	1.90	1.99

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_4157	MSMEG_4157	putative transposase	1.72	2.49	1.68
MSMEG_6613	MSMEG_6613	hypothetical protein	1.72	1.76	1.71
MSMEG_3391	MSMEG_3391	L-carnitine dehydratase,bile acid-inducible protein F	1.72	1.80	1.80
MSMEG_2242	MSMEG_2242	coniferyl aldehyde dehydrogenase, EC Number - 1.2.1.68	1.72	2.33	1.59
MSMEG_0856	MSMEG_0856	DNA-binding response regulator, LuxR family protein	1.72	1.63	1.65
MSMEG_6865	MSMEG_6865	ABC transporter solute binding lipoprotein	1.71	1.95	1.75
MSMEG_0543	MSMEG_0543	hypothetical protein	1.71	1.75	1.67
MSMEG_3909	MSMEG_3909	hypothetical protein	1.71	1.83	1.57
MSMEG_1761	MSMEG_1761	integral membrane protein	1.71	1.68	1.73
MSMEG_5873	MSMEG_5873	hypothetical protein	1.71	1.84	1.82
MSMEG_5137	narI	respiratory nitrate reductase, gamma subunit, EC Number - 1.7.99.4	1.71	2.03	2.59
MSMEG_1142	MSMEG_1142	ABC-transporter integral membrane protein	1.71	1.78	1.59
MSMEG_1986	MSMEG_1986	tartrate dehydrogenase, EC Number - 1.1.1.93	1.71	1.64	1.73
MSMEG_2478	MSMEG_2478	fumarylacetoacetate hydrolase family protein	1.71	1.81	1.68
MSMEG_3846	MSMEG_3846	phosphotransferase enzyme family protein	1.71	1.71	2.03
MSMEG_4127	MSMEG_4127	TnpC protein	1.71	1.85	2.31
MSMEG_6531	MSMEG_6531	hypothetical protein	1.71	1.69	1.78
MSMEG_4732	MSMEG_4732	glycosyl transferase, group 2 family protein, putative	1.71	1.67	1.90
MSMEG_5203	MSMEG_5203	DoxX subfamily protein, putative	1.71	1.91	2.88
MSMEG_3985	MSMEG_3985	integral membrane transport protein	1.71	1.73	2.04
MSMEG_1607	MSMEG_1607	putative tautomerase	1.71	2.09	1.67
MSMEG_1100	MSMEG_1100	dgpf protein	1.71	1.60	1.60
MSMEG_5092	MSMEG_5092	hypothetical protein	1.71	1.56	2.26
MSMEG_6674	MSMEG_6674	transcriptional regulator, LysR family protein	1.71	1.57	1.98
MSMEG_6702	MSMEG_6702	succinate-semialdehyde dehydrogenase [NADP+], EC Number - 1.2.1.16	1.71	1.80	1.67
MSMEG_1298	MSMEG_1298	guanine deaminase, EC Number - 3.5.4.3	1.70	1.61	1.66
MSMEG_3315	MSMEG_3315	hypothetical protein	1.70	2.46	1.73
MSMEG_3703	MSMEG_3703	hypothetical protein	1.70	2.20	1.65
MSMEG_2534	MSMEG_2534	putative carboxylesterase protein	1.70	1.58	2.06
MSMEG_4445	MSMEG_4445	hypothetical protein	1.70	1.64	1.69
MSMEG_3971	MSMEG_3971	hypothetical protein	1.70	1.87	1.76
MSMEG_4393	MSMEG_4393	carboxyvinyl-carboxyphosphonate phosphorylmutase, EC Number - 2.7.8.23	1.70	1.60	2.25
MSMEG_6639	MSMEG_6639	transcriptional regulator, GntR family protein, putative	1.70	1.52	2.00
MSMEG_3664	MSMEG_3664	transporter, monovalent cation:proton antiporter-2 CPA2 family protein	1.70	1.80	1.75
MSMEG_6745	MSMEG_6745	transcriptional regulator, GntR family protein	1.69	1.86	1.59
MSMEG_4855	MSMEG_4855	amidohydrolase family protein	1.69	1.62	1.77
MSMEG_0714	MSMEG_0714	acyl-CoA dehydrogenase, EC Number - 1.3.99.-	1.69	1.98	1.77
MSMEG_4458	MSMEG_4458	transmembrane transport protein	1.69	2.10	2.05
MSMEG_2260	MSMEG_2260	putative transcriptional regulator	1.69	1.89	1.63

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_6781	MSMEG_6781	hypothetical protein	1.69	1.75	1.94
MSMEG_1321	MSMEG_1321	5-carboxymethyl-2-hydroxymuconate delta-isomerase	1.69	1.82	2.21
MSMEG_5012	MSMEG_5012	trans-sialidase, putative	1.69	1.82	1.64
MSMEG_2678	MSMEG_2678	hypothetical protein	1.69	1.94	1.98
MSMEG_5618	MSMEG_5618	acyl-CoA dehydrogenase	1.69	1.68	1.95
MSMEG_6491	MSMEG_6491	aldehyde dehydrogenase	1.69	1.79	1.83
MSMEG_6357	MSMEG_6357	hypothetical protein	1.69	1.86	1.96
MSMEG_6378	MSMEG_6378	senescence marker protein-30	1.69	1.82	1.81
MSMEG_1209	MSMEG_1209	glycosidase	1.69	1.78	1.69
MSMEG_0705	MSMEG_0705	putative permease of the major facilitator superfamily protein	1.69	1.82	2.02
MSMEG_6734	MSMEG_6734	dibenzothiophene desulfurization enzyme A	1.69	1.71	1.61
MSMEG_2098	MSMEG_2098	fumarylacetoacetate hydrolase family protein	1.69	1.63	1.66
MSMEG_1864	MSMEG_1864	transposase	1.69	1.72	2.14
MSMEG_1201	MSMEG_1201	hypothetical protein	1.69	1.93	1.88
MSMEG_4781	MSMEG_4781	hypothetical protein	1.68	1.83	2.81
MSMEG_6108	MSMEG_6108	zinc-binding dehydrogenase	1.68	1.71	2.34
MSMEG_5400	MSMEG_5400	dehydrogenase	1.68	1.55	1.90
MSMEG_4124	MSMEG_4124	hypothetical protein	1.68	1.82	2.12
MSMEG_1894	MSMEG_1894	short chain dehydrogenase	1.68	1.66	1.67
MSMEG_2146	MSMEG_2146	gp15 protein	1.68	1.96	1.63
MSMEG_1856	MSMEG_1856	hypothetical protein	1.68	1.71	1.65
MSMEG_1688	MSMEG_1688	cupin domain protein	1.68	2.05	1.70
MSMEG_0228	MSMEG_0228	adenylate and Guanylate cyclase catalytic domain protein	1.68	1.81	2.18
MSMEG_5974	MSMEG_5974	hypothetical protein	1.68	1.69	1.85
MSMEG_1154	MSMEG_1154	formyl-coenzyme A transferase, EC Number - 2.8.3.16	1.68	1.58	1.83
MSMEG_4864	MSMEG_4864	3-ketosteroid-delta-1-dehydrogenase	1.68	1.83	1.87
MSMEG_4009	MSMEG_4009	vanillate O-demethylase oxidoreductase	1.68	1.87	2.08
MSMEG_3974	MSMEG_3974	N-methylhydantoinase	1.68	1.91	1.79
MSMEG_0675	MSMEG_0675	putative cytochrome P450 144, EC Number - 1.14.-.-	1.68	1.81	1.93
MSMEG_2151	MSMEG_2151	transposase IstA protein, putative, similar to the proteins: MSMEG_1857	1.68	2.27	1.90
MSMEG_4666	MSMEG_4666	myo-inositol 2-dehydrogenase	1.67	1.84	1.69
MSMEG_3584	MSMEG_3584	membrane protein, MmpL family protein	1.67	1.77	1.61
MSMEG_4800	MSMEG_4800	3-oxoacyl-acyl-carrier-protein reductase, putative	1.67	1.63	2.21
MSMEG_4798	MSMEG_4798	L-carnitine dehydratase,bile acid-inducible protein F	1.67	1.62	1.73
MSMEG_5871	MSMEG_5871	HIT family protein	1.67	1.72	2.03
MSMEG_2279	MSMEG_2279	3-hydroxyacyl-CoA dehydrogenase type-2, EC Number - 1.1.1.35 1.1.1.178	1.67	2.17	1.78
MSMEG_4111	MSMEG_4111	alpha-methylacyl-CoA racemase	1.67	1.78	2.02
MSMEG_5628	MSMEG_5628	oxidoreductase, short chain dehydrogenase,reductase family protein	1.67	1.77	1.76
MSMEG_1152	MSMEG_1152	citrate-proton symporter	1.67	1.69	1.94

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_0342	MSMEG_0342	hypothetical protein	1.67	3.01	2.36
MSMEG_3711	MSMEG_3711	hypothetical protein	1.67	1.85	1.78
MSMEG_1219	MSMEG_1219	ABC-type transport system permease protein II	1.67	1.63	1.61
MSMEG_4635	MSMEG_4635	ammonium transporter family protein	1.66	1.69	2.28
MSMEG_4893	MSMEG_4893	Putative neutral zinc metallopeptidase	1.66	1.70	1.75
MSMEG_4451	MSMEG_4451	probable monooxygenase, EC Number - 1.14.13.-	1.66	1.58	1.88
MSMEG_2792	MSMEG_2792	Clp amino terminal domain protein	1.66	2.09	1.73
MSMEG_5347	MSMEG_5347	transporter, major facilitator family protein	1.66	1.77	1.94
MSMEG_3321	MSMEG_3321	ATP,GTP-binding protein	1.66	1.71	1.81
MSMEG_4426	MSMEG_4426	secreted protein	1.66	1.75	1.73
MSMEG_2165	MSMEG_2165	transketolase, N- subunit	1.66	2.20	1.84
MSMEG_6197	MSMEG_6197	diaminopimelate decarboxylase, EC Number - 4.1.1.20	1.66	1.86	1.65
MSMEG_1157	MSMEG_1157	short chain dehydrogenase	1.66	1.72	1.84
MSMEG_2884	MSMEG_2884	cyclohexanone monooxygenase	1.66	1.67	1.87
MSMEG_4019	MSMEG_4019	AMP-dependent synthetase and ligase	1.65	1.56	2.16
MSMEG_2075	hmgA	homogentisate 1,2-dioxygenase, EC Number - 1.13.11.5	1.65	1.65	1.72
MSMEG_1144	MSMEG_1144	virulence factor Mce family protein	1.65	1.55	2.34
MSMEG_3520	MSMEG_3520	TetR-family protein transcriptional regulator	1.65	2.08	1.77
MSMEG_0662	MSMEG_0662	putrescine transport ATP-binding protein PotG	1.65	1.66	1.54
MSMEG_1714	MSMEG_1714	L-ribulose-5-phosphate 4-epimerase UlaF, EC Number - 5.1.3.4	1.65	1.56	1.71
MSMEG_6070	rpmE	50S ribosomal protein L31 type B	1.65	1.90	1.73
MSMEG_2353	MSMEG_2353	secreted protein	1.65	2.25	1.63
MSMEG_1839	MSMEG_1839	alkane 1-monooxygenase, EC Number - 1.14.15.3	1.65	1.67	1.59
MSMEG_5801	MSMEG_5801	hydroxylase	1.65	1.61	2.28
MSMEG_6608	MSMEG_6608	hypothetical protein	1.65	1.86	1.65
MSMEG_2001	MSMEG_2001	sugar transporter	1.65	1.75	1.77
MSMEG_6568	MSMEG_6568	regulatory protein, LuxR, putative	1.65	1.75	1.75
MSMEG_0909	MSMEG_0909	acyl-ACP thioesterase superfamily protein	1.65	1.78	1.56
MSMEG_4068	MSMEG_4068	hypothetical protein	1.65	1.60	1.94
MSMEG_5317	MSMEG_5317	hypothetical protein	1.65	1.66	1.64
MSMEG_5141	MSMEG_5141	nitrate,nitrite transporter protein	1.65	1.90	2.28
MSMEG_5994	MSMEG_5994	putative acyl-CoA dehydrogenase	1.65	1.62	1.79
MSMEG_1745	MSMEG_1745	transcriptional regulator	1.65	1.53	1.70
MSMEG_0208	MSMEG_0208	ribonuclease	1.65	1.58	1.81
MSMEG_1118	MSMEG_1118	amino acid permease	1.65	1.54	1.62
MSMEG_0188	MSMEG_0188	MarR-family protein transcriptional regulator	1.64	1.83	1.74
MSMEG_6851	MSMEG_6851	phosphatidylethanolamine-binding protein	1.64	1.79	1.69
MSMEG_6466	MSMEG_6466	aquaporin Z	1.64	1.84	1.94
MSMEG_3716	MSMEG_3716	hypothetical protein	1.64	2.00	1.83
MSMEG_2867	MSMEG_2867	3-ketosteroid-delta-1-dehydrogenase	1.64	1.67	2.01
MSMEG_6688	MSMEG_6688	regulatory protein	1.64	1.60	1.89

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_5288	MSMEG_5288	putative serine,threonine protein kinase	1.64	1.83	1.99
MSMEG_6603	MSMEG_6603	NADH:flavin oxidoreductase,nadh oxidase	1.64	1.70	1.71
MSMEG_2215	MSMEG_2215	AMP-binding enzyme	1.64	1.77	1.66
MSMEG_4837	MSMEG_4837	amidohydrolase 2	1.63	1.59	1.84
MSMEG_4828	MSMEG_4828	alcohol dehydrogenase, zinc-binding	1.63	2.05	1.73
MSMEG_4446	lpdA	dihydrolipoamide dehydrogenase, EC Number - 1.8.1.4	1.63	1.84	1.75
MSMEG_1461	MSMEG_1461	5-oxovalerate dehydrogenase	1.63	1.59	1.66
MSMEG_4436	MSMEG_4436	transketolase, EC Number - 2.2.1.1	1.63	1.74	2.31
MSMEG_2347	MSMEG_2347	phytoene dehydrogenase, EC Number - 1.14.99.-	1.63	1.78	1.69
MSMEG_5759	MSMEG_5759	pe family protein	1.63	1.66	1.87
MSMEG_4888	MSMEG_4888	periplasmic binding proteins and sugar binding domain of the LacI family protein, putative	1.63	1.67	1.65
MSMEG_4413	MSMEG_4413	hypothetical protein	1.63	1.66	1.98
MSMEG_4863	MSMEG_4863	hypothetical protein	1.63	1.74	1.96
MSMEG_0894	MSMEG_0894	dihydrodipicolinate reductase	1.62	1.76	1.68
MSMEG_3715	MSMEG_3715	linear gramicidin synthetase subunit C, EC Number - 5.1.1.- 5.1.1.- 5.1.1.-	1.62	1.80	2.12
MSMEG_3427	MSMEG_3427	metal dependent hydrolase	1.62	1.65	2.44
MSMEG_1988	MSMEG_1988	hypothetical protein	1.62	1.66	1.58
MSMEG_4661	MSMEG_4661	sugar kinase, ribokinase family protein	1.62	2.04	2.27
MSMEG_6874	MSMEG_6874	aldehyde dehydrogenase	1.62	1.79	1.77
MSMEG_6770	MSMEG_6770	hypothetical protein	1.62	1.88	1.74
MSMEG_5962	MSMEG_5962	glycosyl transferase, family protein 2	1.62	1.75	2.08
MSMEG_2058	nuoF	NADH-quinone oxidoreductase, F subunit, EC Number - 1.6.99.5	1.62	1.53	1.81
MSMEG_1072	speB	agmatinase, EC Number - 3.5.3.11	1.61	1.66	1.88
MSMEG_4562	MSMEG_4562	ABC Fe3+-siderophores transporter, periplasmic binding protein	1.61	1.83	1.76
MSMEG_6048	MSMEG_6048	cobalamin synthesis protein,P47K	1.61	1.92	1.69
MSMEG_4075	MSMEG_4075	CoA-binding protein	1.61	1.98	2.06
MSMEG_1138	MSMEG_1138	alcohol dehydrogenase 1, EC Number - 1.1.1.1	1.61	1.79	1.69
MSMEG_1253	MSMEG_1253	hypothetical protein	1.61	1.58	1.97
MSMEG_2345	MSMEG_2345	dehydrogenase	1.61	1.80	1.63
MSMEG_5652	MSMEG_5652	alpha,beta hydrolase fold	1.61	1.81	1.72
MSMEG_2482	MSMEG_2482	hypothetical protein	1.61	1.55	1.66
MSMEG_0609	MSMEG_0609	helix-turn-helix domain protein	1.60	1.81	1.74
MSMEG_2634	MSMEG_2634	hypothetical protein	1.60	1.66	1.71
MSMEG_1870	MSMEG_1870	hypothetical protein	1.60	1.63	1.61
MSMEG_2892	MSMEG_2892	pigment production hydroxylase, EC Number - 1.-.-.-	1.60	1.65	1.95
MSMEG_1096	MSMEG_1096	urease accessory protein UreD, putative	1.60	1.65	1.75
MSMEG_2012	hisD	histidinol dehydrogenase, EC Number - 1.1.1.23	1.60	1.74	1.90
MSMEG_0183	MSMEG_0183	hypothetical protein	1.60	1.75	2.11
MSMEG_4060	MSMEG_4060	haloacid dehalogenase, type II	1.60	1.97	1.65
MSMEG_6760	MSMEG_6760	hypothetical protein	1.60	1.79	1.87

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_3701	MSMEG_3701	hypothetical protein	1.60	1.59	1.63
MSMEG_4030	MSMEG_4030	hypothetical protein	1.60	1.63	1.75
MSMEG_5462	rarD	RarD protein	1.60	1.63	1.82
MSMEG_0322	MSMEG_0322	transcriptional regulator	1.60	1.85	2.15
MSMEG_2857	MSMEG_2857	virulence factor Mce family protein	1.59	1.76	1.83
MSMEG_2601	pcaH	protocatechuate 3,4-dioxygenase, beta subunit, EC Number - 1.13.11.3	1.59	1.61	1.71
MSMEG_4038	MSMEG_4038	vanillin dehydrogenase	1.59	1.68	1.76
MSMEG_2121	MSMEG_2121	multiphosphoryl transfer protein MTP, EC Number - 2.7.3.9 2.7.1.-	1.59	1.83	1.74
MSMEG_6146	MSMEG_6146	ISMsm3, transposase	1.59	1.93	1.60
MSMEG_0453	aroK	shikimate kinase, EC Number - 2.7.1.71	1.59	1.81	1.87
MSMEG_6765	MSMEG_6765	ABC-2 type transporter superfamily protein	1.59	1.61	2.26
MSMEG_4799	MSMEG_4799	aldehyde dehydrogenase NAD family protein, EC Number - 1.2.1.-	1.59	1.71	2.09
MSMEG_2873	MSMEG_2873	hypothetical protein	1.59	1.74	1.96
MSMEG_6708	MSMEG_6708	epoxide hydrolase	1.59	2.99	1.94
MSMEG_4000	MSMEG_4000	hypothetical ABC transporter ATP-binding protein YphE	1.59	1.83	2.19
MSMEG_2852	MSMEG_2852	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	1.59	1.70	1.74
MSMEG_3996	hydA	dihydropyrimidinase, EC Number - 3.5.2.2	1.58	1.83	1.87
MSMEG_4338	MSMEG_4338	possible transcriptional regulatory protein	1.58	1.80	1.91
MSMEG_1145	MSMEG_1145	virulence factor Mce family protein	1.58	1.58	1.66
MSMEG_3678	MSMEG_3678	hypothetical protein	1.58	1.85	2.01
MSMEG_2702	hypD	hydrogenase expression,formation protein HypD	1.58	1.81	1.71
MSMEG_5908	MSMEG_5908	acyl-CoA synthetase	1.57	1.69	2.18
MSMEG_3312	MSMEG_3312	hemerythrin HHE cation binding domain subfamily protein, putative	1.57	1.65	2.04
MSMEG_2602	pcaG	protocatechuate 3,4-dioxygenase, alpha subunit, EC Number - 1.13.11.3	1.57	1.82	2.05
MSMEG_4460	MSMEG_4460	acetolactate synthase	1.57	1.91	2.06
MSMEG_1129	MSMEG_1129	D-amino-acid dehydrogenase	1.57	1.78	1.65
MSMEG_2241	MSMEG_2241	acyl-CoA synthetase	1.57	1.84	1.67
MSMEG_2711	hypF	[NiFe] hydrogenase maturation protein HypF	1.57	1.71	1.64
MSMEG_4814	MSMEG_4814	hypothetical protein	1.57	1.71	1.71
MSMEG_4055	MSMEG_4055	short-chain dehydrogenase,reductase SDR	1.57	1.66	1.88
MSMEG_2479	MSMEG_2479	hypothetical protein	1.56	1.98	1.63
MSMEG_3700	MSMEG_3700	peroxidase	1.56	1.52	1.81
MSMEG_1775	MSMEG_1775	cytochrome P450 monooxygenase	1.56	1.95	1.58
MSMEG_3842	MSMEG_3842	putative esterase	1.56	1.71	1.71
MSMEG_4203	MSMEG_4203	peptidase S8 and S53, subtilisin, kexin, sedolisin	1.56	1.63	1.66
MSMEG_1960	MSMEG_1960	hypothetical protein	1.56	1.63	1.77
MSMEG_4004	MSMEG_4004	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	1.55	1.80	1.83
MSMEG_1407	MSMEG_1407	hypothetical protein	1.54	1.57	2.22
MSMEG_3969	MSMEG_3969	hypothetical protein	1.54	1.66	2.06
MSMEG_2145	MSMEG_2145	hypothetical protein	1.54	1.65	1.70

Probe Name	Gene Name	Description	Fold change		
			Wayne	DPI	AA
MSMEG_2077	MSMEG_2077	acyl-CoA dehydrogenase, C- domain protein	1.53	1.75	1.85

4.3.1.3 Common Genes Down-regulated Under Three Different Conditions

A total of 1362 genes were found to be down-regulated under all three conditions above 1.5 fold change. Amongst these genes, total 917 genes were found to have 2 fold expression changes. Below is the list of down-regulated genes above 2 fold.

Table 4.3: List of genes down-regulated under all three conditions (Expression ratio above 2 fold)

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_4545	MSMEG_4545	bacterial extracellular solute-binding protein, family protein 5	58.83	56.80	7.23
MSMEG_4086	ssuD	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.-	46.94	36.99	19.83
MSMEG_1193	MSMEG_1193	TROVE domain protein	40.92	40.12	28.08
MSMEG_2736	MSMEG_2736	GTP-binding protein	36.65	33.46	27.50
MSMEG_1524	rpoA	DNA-directed RNA polymerase, alpha subunit, EC Number - 2.7.7.6	35.65	51.35	24.60
MSMEG_3404	MSMEG_3404	HNH endonuclease domain protein	34.66	30.08	16.04
MSMEG_5646	MSMEG_5646	hypothetical protein	34.62	28.88	11.48
MSMEG_1522	rpsK	30S ribosomal protein S11	33.99	38.81	26.37
MSMEG_4085	MSMEG_4085	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.-	33.29	95.85	13.96
MSMEG_1523	rpsD	30S ribosomal protein S4	30.03	39.38	21.02
MSMEG_3669	MSMEG_3669	hypothetical protein	29.54	24.20	9.45
MSMEG_4084	MSMEG_4084	putative acyl-CoA dehydrogenase	29.39	29.48	11.26
MSMEG_1651	metX	homoserine O-acetyltransferase, EC Number - 2.3.1.31	29.20	27.77	21.91
MSMEG_4236	mraZ	hypothetical protein	28.90	30.34	11.88
MSMEG_1401	tuf	elongation factor Tu	28.61	32.89	22.41
MSMEG_4083	MSMEG_4083	putative monooxygenase	28.58	27.78	9.05
MSMEG_2580	ispG	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase, EC Number - 1.17.4.3	28.30	33.79	18.63
MSMEG_0546	MSMEG_0546	hypothetical protein	27.09	30.27	6.80
MSMEG_3056	MSMEG_3056	ABC transporter ATP-binding protein	25.36	24.65	15.18
MSMEG_1442	rpsC	30S ribosomal protein S3	24.19	31.35	20.12
MSMEG_3137	MSMEG_3137	oxidoreductase	23.47	27.13	16.02
MSMEG_1466	rplX	50S ribosomal protein L24	22.88	24.82	19.09
MSMEG_4552	ssuD	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.-	22.58	18.75	9.81
MSMEG_1441	MSMEG_1441	50S ribosomal protein L22	22.31	24.94	20.08

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_1065	MSMEG_1065	hypothetical protein	22.04	20.39	16.83
MSMEG_2450	MSMEG_2450	hypothetical protein	21.85	20.97	13.26
MSMEG_4586	MSMEG_4586	ABC nitrate,sulfonate,bicarbonate family protein transporter, periplasmic ligand binding protein	21.21	20.85	7.02
MSMEG_1064	MSMEG_1064	phosphate,sulphate permease	21.12	20.34	18.94
MSMEG_4624	rpmA	50S ribosomal protein L27	20.91	19.85	18.98
MSMEG_4087	MSMEG_4087	major facilitator superfamily protein	20.61	18.53	13.23
MSMEG_4528	MSMEG_4528	phosphoadenosine phosphosulfate reductase, EC Number - 1.8.4.8	19.48	24.03	16.00
MSMEG_0172	MSMEG_0172	probable conserved transmembrane protein, putative	19.47	19.78	16.28
MSMEG_6669	MSMEG_6669	ABC transporter, permease protein	18.96	23.20	21.11
MSMEG_1652	MSMEG_1652	O-acetylhomoserine aminocarboxypropyltransferase	18.83	18.88	16.45
MSMEG_3751	MSMEG_3751	hemolysin A	18.71	17.48	12.55
MSMEG_3662	MSMEG_3662	mannose-binding lectin	18.67	20.12	4.98
MSMEG_1650	MSMEG_1650	methyltransferase type 11	18.41	66.43	16.18
MSMEG_4094	MSMEG_4094	acyl-CoA dehydrogenase	17.93	17.11	4.70
MSMEG_3833	MSMEG_3833	30S ribosomal protein S1	17.33	17.98	13.00
MSMEG_1516	MSMEG_1516	thioredoxin reductase	17.02	17.93	13.35
MSMEG_1017	MSMEG_1017	glutaredoxin, similar to the proteins: MSMEG_2297	16.97	19.94	12.12
MSMEG_1036	MSMEG_1036	hypothetical protein	16.68	14.37	11.23
MSMEG_2045	MSMEG_2045	monoxygenase	16.54	14.99	10.43
MSMEG_1400	MSMEG_1400	30S ribosomal protein S7	16.14	24.42	11.35
MSMEG_2316	MSMEG_2316	hypothetical protein	16.07	13.47	11.20
MSMEG_0965	MSMEG_0965	porin	16.02	11.82	6.64
MSMEG_4260	MSMEG_4260	cytoChrome c oxidase subunit 3, EC Number - 1.9.3.1	15.95	15.72	8.87
MSMEG_3950	MSMEG_3950	universal stress protein family protein	15.80	14.16	7.53
MSMEG_5525	sucC	succinyl-CoA synthetase, beta subunit, EC Number - 6.2.1.5	15.72	16.87	12.23
MSMEG_3126	MSMEG_3126	SUF system FeS assembly protein, NifU family protein	15.71	17.16	13.09
MSMEG_2352	etfA	electron transfer flavoprotein, alpha subunit	15.53	17.67	15.20
MSMEG_5268	MSMEG_5268	hypothetical protein	15.52	13.90	11.07
MSMEG_1437	rplD	50S ribosomal protein L4	15.35	16.78	12.82
MSMEG_3085	pgk	phosphoglycerate kinase, EC Number - 2.7.2.3	15.29	18.21	11.04
MSMEG_2846	MSMEG_2846	ABC transporter permease protein	15.25	14.53	10.32
MSMEG_1436	rplC	50S ribosomal protein L3	15.02	17.09	14.25
MSMEG_4588	MSMEG_4588	ABC nitrate,sulfonate,bicarbonate family protein transporter, inner membrane subunit	14.80	12.48	8.36
MSMEG_0586	MSMEG_0586	stas domain, putative	14.77	15.77	14.84
MSMEG_1521	rpsM	30S ribosomal protein S13	14.76	134.70	13.65
MSMEG_5246	MSMEG_5246	hypothetical protein	14.76	17.71	8.44
MSMEG_3138	trx	thioredoxin	14.67	17.07	9.82
MSMEG_2847	MSMEG_2847	bacterial extracellular solute-binding protein, family protein 5	14.51	14.46	9.50
MSMEG_0939	MSMEG_0939	Ppx,GppA phosphatase family protein	14.44	16.98	10.75

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_1471	rplR	50S ribosomal protein L18	14.23	13.81	14.43
MSMEG_3102	tal	transaldolase, EC Number - 2.2.1.2	14.21	16.95	10.68
MSMEG_1399	rpsG	30S ribosomal protein S7	14.12	17.83	12.85
MSMEG_1468	rpsN	ribosomal protein S14p,S29e	13.94	18.07	14.30
MSMEG_5483	MSMEG_5483	porin	13.93	11.88	5.88
MSMEG_2441	lepB	signal peptidase I, EC Number - 3.4.21.89	13.85	20.99	7.29
MSMEG_1438	rplW	50S ribosomal protein L23	13.83	16.04	11.45
MSMEG_4587	MSMEG_4587	putative aliphatic sulfonates transport ATP-binding protein SsuB	13.80	15.11	7.99
MSMEG_1440	rpsS	30S ribosomal protein S19	13.79	18.36	12.15
MSMEG_4590	ssuD	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.-	13.73	12.45	7.04
MSMEG_2585	MSMEG_2585	hypothetical protein	13.63	13.95	9.56
MSMEG_5647	MSMEG_5647	hypothetical protein	13.62	14.42	14.81
MSMEG_1367	rpoB	DNA-directed RNA polymerase, beta subunit, EC Number - 2.7.7.6	13.48	14.53	11.25
MSMEG_2656	gpsI	guanosine pentaphosphate synthetase I,polyribonucleotide nucleotidyltransferase, EC Number - 2.7.-.- 2.7.7.8	13.48	13.26	13.74
MSMEG_1600	MSMEG_1600	hypothetical protein	13.46	15.68	9.03
MSMEG_4290	glnA	glutamine synthetase, type I, EC Number - 6.3.1.2	13.38	15.21	11.05
MSMEG_5524	sucD	succinyl-CoA synthetase, alpha subunit, EC Number - 6.2.1.5	13.31	60.61	11.53
MSMEG_1483	MSMEG_1483	preprotein translocase subunit SecY	13.27	16.61	12.65
MSMEG_1443	rplP	50S ribosomal protein L16	13.21	34.37	8.48
MSMEG_4592	MSMEG_4592	acyl-CoA dehydrogenase family protein	13.18	11.97	9.60
MSMEG_1445	MSMEG_1445	30S ribosomal protein S17	13.03	16.20	11.19
MSMEG_4401	MSMEG_4401	phosphonoacetaldehyde hydrolase	12.90	10.44	11.18
MSMEG_2756	MSMEG_2756	hypothetical protein	12.90	28.28	12.25
MSMEG_2372	ilvB	acetolactate synthase, large subunit, biosynthetic type, EC Number - 2.2.1.6	12.71	12.20	11.21
MSMEG_4098	MSMEG_4098	ABC transporter ATP-binding protein	12.60	11.31	6.86
MSMEG_4589	MSMEG_4589	rhodanese domain protein,cystathionine beta-lyase	12.53	13.49	9.13
MSMEG_4063	MSMEG_4063	amidohydrolase family protein	12.53	13.71	8.84
MSMEG_1398	rpsL	30S ribosomal protein S12	12.50	15.12	11.26
MSMEG_0932	MSMEG_0932	ROK family protein	12.42	11.72	9.53
MSMEG_1953	MSMEG_1953	transcription factor WhiB	12.39	10.72	10.96
MSMEG_2519	rpsB	30S ribosomal protein S2	12.31	12.57	10.26
MSMEG_5518	MSMEG_5518	antigen 34 kDa	12.25	14.82	10.40
MSMEG_3206	hisC	histidinol-phosphate aminotransferase, EC Number - 2.6.1.9	12.24	10.55	10.79
MSMEG_1467	MSMEG_1467	50S ribosomal protein L5	11.92	16.87	12.51
MSMEG_2520	tsf	elongation factor Ts	11.89	10.92	10.44
MSMEG_3792	rpmI	50S ribosomal protein L35	11.87	15.15	9.38
MSMEG_3125	MSMEG_3125	cysteine desulfurase, EC Number - 2.8.1.7 4.4.1.16	11.78	11.19	9.89
MSMEG_5187	MSMEG_5187	tetracycline-resistance determinant TetV	11.77	11.15	10.32
MSMEG_3896	MSMEG_3896	hypothetical protein	11.68	13.52	8.12

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_1520	rpmJ	50S ribosomal protein L36	11.67	13.60	11.11
MSMEG_0116	MSMEG_0116	taurine import ATP-binding protein TauB, EC Number - 3.6.3.36	11.67	10.39	12.26
MSMEG_3778	pheS	phenylalanyl-tRNA synthetase, alpha subunit, EC Number - 6.1.1.20	11.64	7.88	8.70
MSMEG_2755	MSMEG_2755	hypothetical protein	11.63	4.10	8.67
MSMEG_0774	MSMEG_0774	acetyltransferase	11.61	12.89	10.87
MSMEG_0520	MSMEG_0520	porin	11.56	5.06	4.86
MSMEG_1930	MSMEG_1930	DEAD,DEAH box helicase	11.54	11.94	8.30
MSMEG_5789	MSMEG_5789	putative thiosulfate sulfurtransferase, EC Number - 2.8.1.1	11.43	13.48	9.83
MSMEG_3755	rrfA	5S ribosomal RNA	11.41	5.52	7.59
MSMEG_1368	rpoC	DNA-directed RNA polymerase, beta subunit, EC Number - 2.7.7.6	11.24	11.93	10.31
MSMEG_4262	MSMEG_4262	ubiquinol-cytochrome c reductase iron-sulfur subunit	11.23	11.00	8.90
MSMEG_1473	rpmD	ribosomal protein L30	11.23	14.01	10.47
MSMEG_5607	MSMEG_5607	hypothetical protein	11.11	10.83	13.34
MSMEG_5264	MSMEG_5264	hypothetical protein	10.96	10.94	8.88
MSMEG_1485	map	methionine aminopeptidase, type I, EC Number - 3.4.11.18	10.77	11.23	9.78
MSMEG_1037	MSMEG_1037	alcohol dehydrogenase, zinc-containing, EC Number - 1.1.1.2, similar to the proteins: MSMEG_2317	10.77	15.41	12.20
MSMEG_5781	pstC	phosphate ABC transporter, permease protein PstC	10.77	10.26	11.40
MSMEG_4327	MSMEG_4327	3-oxoacyl-[acyl-carrier-protein] synthase 1, EC Number - 2.3.1.41	10.76	11.41	7.14
MSMEG_4937	atpG	ATP synthase F1, gamma subunit, EC Number - 3.6.3.14	10.75	10.34	10.13
MSMEG_0880	groL	chaperonin GroEL	10.73	17.76	10.54
MSMEG_2651	MSMEG_2651	alkanesulfonate monooxygenase family protein	10.67	8.95	9.34
MSMEG_0559	MSMEG_0559	hypothetical protein	10.67	24.42	10.38
MSMEG_5263	MSMEG_5263	transcription elongation factor GreA	10.67	10.27	8.59
MSMEG_2795	MSMEG_2795	transmembrane transport protein	10.63	9.74	10.04
MSMEG_4547	MSMEG_4547	acyl-CoA dehydrogenase family protein	10.60	8.64	5.74
MSMEG_1345	MSMEG_1345	transcription antitermination protein NusG	10.53	9.35	8.70
MSMEG_4082	MSMEG_4082	monoxygenase	10.53	12.37	7.07
MSMEG_5245	MSMEG_5245	universal stress protein family protein	10.52	11.50	7.81
MSMEG_2614	MSMEG_2614	ElaA protein	10.52	11.32	6.90
MSMEG_1444	rpmC	50S ribosomal protein L29	10.47	47.79	6.91
MSMEG_2351	etfB	electron transfer flavoprotein, beta subunit	10.36	12.00	9.32
MSMEG_2628	infB	translation initiation factor IF-2	10.27	12.04	10.45
MSMEG_4326	acpP	acyl carrier protein	10.25	12.30	5.15
MSMEG_2629	rbfA	ribosome-binding factor A	10.24	9.56	9.38
MSMEG_1525	MSMEG_1525	50S ribosomal protein L17	10.10	12.02	8.90
MSMEG_2626	MSMEG_2626	hypothetical protein	10.09	12.14	7.60
MSMEG_4756	acpS	holo-acyl-carrier-protein synthase, EC Number - 2.7.8.7	10.05	10.07	8.69
MSMEG_1439	rplB	50S ribosomal protein L2	10.04	12.49	9.17

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_1012	MSMEG_1012	alkaline phosphatase, EC Number - 3.1.3.1, similar to the proteins: MSMEG_2292	10.00	9.58	9.57
MSMEG_1472	rpsE	30S ribosomal protein S5	9.96	10.98	10.61
MSMEG_1066	MSMEG_1066	hypothetical protein	9.93	10.08	9.64
MSMEG_5308	MSMEG_5308	hypothetical protein	9.85	10.01	8.94
MSMEG_4929	rrfB	5S ribosomal RNA	9.85	4.71	6.61
MSMEG_5529	MSMEG_5529	hypothetical protein	9.83	8.43	9.05
MSMEG_0557	MSMEG_0557	hypothetical protein	9.66	78.53	5.75
MSMEG_4168	MSMEG_4168	propionate CoA-transferase	9.57	10.39	7.94
MSMEG_1470	MSMEG_1470	50S ribosomal protein L6	9.52	9.84	9.21
MSMEG_2920	MSMEG_2920	aldo,keto reductase	9.43	8.96	7.01
MSMEG_5824	purL	phosphoribosylformylglycinamidine synthase II, EC Number - 6.3.5.3	9.42	9.11	7.78
MSMEG_4336	MSMEG_4336	hypothetical protein	9.39	10.28	6.67
MSMEG_0649	MSMEG_0649	phosphonate-binding periplasmic protein	9.28	9.71	8.69
MSMEG_5489	rpmF	50S ribosomal protein L32	9.28	9.01	7.54
MSMEG_5790	MSMEG_5790	SseC protein	9.24	7.77	8.39
MSMEG_1018	MSMEG_1018	glutaredoxin, similar to the proteins: MSMEG_2298	9.24	16.29	9.78
MSMEG_5809	MSMEG_5809	lipoprotein YaeC, putative	9.23	9.50	4.94
MSMEG_3621	ndh	NADH dehydrogenase, EC Number - 1.6.99.3	9.20	19.14	8.42
MSMEG_4942	atpB	ATP synthase F0, A subunit, EC Number - 3.6.3.14	9.15	9.91	7.29
MSMEG_2630	MSMEG_2630	DHH family protein	9.08	8.06	8.20
MSMEG_1469	rpsH	30S ribosomal protein S8	9.01	8.33	7.70
MSMEG_3668	MSMEG_3668	acyl-CoA dehydrogenase	8.98	8.32	7.44
MSMEG_1435	rpsJ	ribosomal protein S10	8.98	10.54	8.74
MSMEG_4625	rplU	50S ribosomal protein L21	8.97	8.80	8.47
MSMEG_3771	argR	arginine repressor	8.96	7.91	7.50
MSMEG_5081	MSMEG_5081	hypothetical protein	8.91	9.16	9.14
MSMEG_4536	MSMEG_4536	hypothetical protein	8.87	14.51	7.98
MSMEG_4494	MSMEG_4494	hypothetical protein	8.87	9.13	6.65
MSMEG_5782	pstS	phosphate ABC transporter, phosphate-binding protein PstS	8.87	7.82	8.34
MSMEG_4890	ahpD	alkylhydroperoxidase, AhpD family protein	8.76	7.12	6.19
MSMEG_2430	ffh	signal recognition particle protein	8.72	8.83	6.78
MSMEG_1040	ctaD	cytochrome c oxidase, subunit I, EC Number - 1.9.3.1, similar to the proteins: MSMEG_2320	8.72	9.70	8.15
MSMEG_2044	MSMEG_2044	integral membrane transport protein	8.69	8.39	7.67
MSMEG_2435	MSMEG_2435	30S ribosomal protein S16	8.65	7.45	7.85
MSMEG_4261	MSMEG_4261	ubiquinol-cytochrome c reductase cytochrome c subunit	8.63	561.27	7.93
MSMEG_2921	MSMEG_2921	NADPH-flavin oxidoreductase, EC Number - 1.6.99.-	8.63	95.36	7.53
MSMEG_3794	MSMEG_3794	hypothetical protein	8.62	8.19	5.68
MSMEG_2844	MSMEG_2844	ABC transporter, ATP-binding protein	8.61	8.41	5.16
MSMEG_4257	MSMEG_4257	hypothetical protein	8.58	8.89	7.55
MSMEG_1738	MSMEG_1738	probable conserved transmembrane protein	8.58	8.51	4.40

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_3850	MSMEG_3850	alkanesulfonate monooxygenase family protein	8.57	7.62	6.42
MSMEG_3545	MSMEG_3545	hypothetical protein	8.54	8.19	5.85
MSMEG_4593	MSMEG_4593	acyl-CoA dehydrogenase	8.53	8.05	5.98
MSMEG_1346	rplK	50S ribosomal protein L11	8.51	10.30	8.25
MSMEG_2356	MSMEG_2356	acyltransferase	8.51	7.88	9.29
MSMEG_6253	MSMEG_6253	fur family protein transcriptional regulator	8.49	9.49	7.40
MSMEG_5051	MSMEG_5051	major facilitator superfamily protein	8.38	8.07	7.79
MSMEG_4549	MSMEG_4549	amino acid permease-associated region, putative	8.38	7.84	5.49
MSMEG_4268	MSMEG_4268	cytoChrome c oxidase subunit 2, EC Number - 1.9.3.1	8.37	8.76	6.20
MSMEG_6084	MSMEG_6084	base excision DNA repair protein, HhH-GPD family protein	8.29	8.05	7.33
MSMEG_4557	MSMEG_4557	ABC transporter, ATP-binding protein	8.26	7.50	8.28
MSMEG_3902	MSMEG_3902	ATPase, AAA family protein	8.23	9.81	6.23
MSMEG_4328	MSMEG_4328	3-oxoacyl-[acyl-carrier-protein] synthase 2, EC Number - 2.3.1.41	8.22	10.70	4.49
MSMEG_5532	MSMEG_5532	FAD dependent oxidoreductase	8.20	6.36	5.66
MSMEG_1831	MSMEG_1831	Transcription factor WhiB	8.11	21.12	7.47
MSMEG_4500	MSMEG_4500	hypothetical protein	8.08	7.66	6.34
MSMEG_1886	MSMEG_1886	Fatty acid desaturase	8.06	35.26	5.95
MSMEG_5779	pstB	phosphate ABC transporter, ATP-binding protein, EC Number - 3.6.3.27	8.04	7.45	7.72
MSMEG_4932	murA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase, EC Number - 2.5.1.7	8.03	8.81	7.76
MSMEG_6091	MSMEG_6091	negative regulator of genetic competence ClpC,mecB	7.96	14.65	7.81
MSMEG_5132	typA	GTP-binding protein TypA,BipA	7.93	7.81	6.80
MSMEG_2696	MSMEG_2696	putative conserved membrane alanine rich protein	7.88	7.09	9.00
MSMEG_5808	MSMEG_5808	binding-protein-dependent transport systems inner membrane component	7.84	7.25	4.12
MSMEG_4595	MSMEG_4595	probable serine,threonine-protein kinase PknH, putative, EC Number - 2.7.11.1	7.83	6.76	6.08
MSMEG_0114	MSMEG_0114	extracellular solute-binding protein, family protein 3	7.82	7.46	7.27
MSMEG_5270	MSMEG_5270	cystathionine beta-synthase, EC Number - 4.2.1.22	7.79	20.08	6.65
MSMEG_4902	MSMEG_4902	metal-dependent hydrolase of the beta-lactamase superfamily protein III	7.72	7.81	6.39
MSMEG_4267	MSMEG_4267	probable Cytochrome c oxidase polypeptide 4, EC Number - 1.9.3.1	7.71	9.27	7.19
MSMEG_6945	rnpA	ribonuclease P protein component, EC Number - 3.1.26.5	7.70	7.29	7.34
MSMEG_4222	ftsZ	cell division protein FtsZ	7.64	29.92	7.70
MSMEG_6257	MSMEG_6257	asparate kinase, monofunctional class, EC Number - 2.7.2.4	7.60	7.71	7.74
MSMEG_1556	rplM	50S ribosomal protein L13	7.58	8.79	6.54
MSMEG_3057	MSMEG_3057	ABC-type metal ion transport system, permease component	7.57	7.00	5.53
MSMEG_2385	MSMEG_2385	Glu tRNA	7.54	6.32	4.83
MSMEG_2579	MSMEG_2579	zinc metalloprotease	7.51	7.94	7.33
MSMEG_3175	MSMEG_3175	ribosomal large subunit pseudouridine synthase D, EC Number - 5.4.99.-	7.44	8.39	6.65
MSMEG_4099	MSMEG_4099	ABC transporter permease protein	7.42	7.36	5.61

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_3124	sufC	FeS assembly ATPase SufC	7.39	6.89	7.21
MSMEG_2449	mmsA	methylmalonate-semialdehyde dehydrogenase, EC Number - 1.2.1.27	7.36	5.21	4.94
MSMEG_0936	senX3	sensor histidine kinase SenX3, EC Number - 2.7.3.-	7.35	6.29	7.82
MSMEG_4630	valS	valyl-tRNA synthetase, EC Number - 6.1.1.9	7.34	7.49	6.58
MSMEG_1347	rplA	50S ribosomal protein L1	7.34	6.75	6.90
MSMEG_3791	rplT	50S ribosomal protein L20	7.32	6.91	6.99
MSMEG_2845	MSMEG_2845	ABC transporter permease protein	7.30	5.81	5.45
MSMEG_5485	MSMEG_5485	molybdopterin biosynthesis protein	7.28	7.96	8.21
MSMEG_3205	hisD	histidinol dehydrogenase, EC Number - 1.1.1.23	7.26	6.52	7.01
MSMEG_3870	MSMEG_3870	alpha-ketoglutarate-dependent taurine dioxygenase, EC Number - 1.14.11.17	7.21	8.65	5.90
MSMEG_6668	MSMEG_6668	ABC transporter, periplasmic substrate-binding protein, putative	7.20	7.67	7.49
MSMEG_4272	MSMEG_4272	HesB,YadR,YfhF family protein	7.19	13.13	7.38
MSMEG_1582	groS	co-chaperonin GroES	7.17	7.81	6.73
MSMEG_4551	MSMEG_4551	monoxygenase	7.16	6.14	5.73
MSMEG_1341	MSMEG_1341	MaoC family protein	7.08	6.44	6.64
MSMEG_5071	MSMEG_5071	hypothetical protein	7.02	14.10	8.05
MSMEG_1862	MSMEG_1862	transposase	6.94	8.01	6.26
MSMEG_1618	MSMEG_1618	hypothetical protein	6.94	9.95	5.93
MSMEG_1465	rplN	ribosomal protein L14	6.92	20.13	6.03
MSMEG_4748	MSMEG_4748	hypothetical protein	6.91	6.61	5.17
MSMEG_5531	MSMEG_5531	ABC transporter ATP-binding protein	6.90	7.08	6.43
MSMEG_2358	trmU	tRNA 5-methylaminomethyl-2-thiouridylate-methyltransferase, EC Number - 2.1.1.61	6.88	6.39	6.59
MSMEG_1519	infA	translation initiation factor IF-1	6.85	8.10	5.82
MSMEG_6256	asd	aspartate-semialdehyde dehydrogenase, EC Number - 1.2.1.11	6.85	6.70	6.99
MSMEG_5780	pstA	phosphate ABC transporter, permease protein PstA	6.81	7.15	7.19
MSMEG_6135	MSMEG_6135	hypothetical protein	6.80	13.92	6.50
MSMEG_5533	MSMEG_5533	4Fe-4S ferredoxin, iron-sulfur binding	6.77	5.75	5.32
MSMEG_1484	MSMEG_1484	adenylate kinase, EC Number - 2.7.4.3	6.75	7.77	6.40
MSMEG_3811	MSMEG_3811	universal stress protein family protein, putative	6.72	6.79	5.73
MSMEG_3689	MSMEG_3689	sodium:solute symporter	6.70	7.17	6.50
MSMEG_1254	MSMEG_1254	DEAD,DEAH box helicase	6.62	9.68	4.87
MSMEG_3123	sufD	FeS assembly protein SufD	6.62	6.10	6.31
MSMEG_2613	mqo	malate:quinone-oxidoreductase, EC Number - 1.1.99.16	6.59	7.00	6.27
MSMEG_4499	MSMEG_4499	hypothetical protein	6.57	6.54	5.15
MSMEG_5279	MSMEG_5279	hypothetical protein	6.55	6.35	6.03
MSMEG_1526	MSMEG_1526	probable cutinase ,, EC Number - 3.1.1.74	6.48	6.54	6.06
MSMEG_4276	ilvE	branched-chain amino acid aminotransferase, EC Number - 2.6.1.42	6.44	6.85	6.48
MSMEG_4541	MSMEG_4541	ABC transporter, ATP-binding protein	6.44	6.10	5.78
MSMEG_2657	MSMEG_2657	peptidase, M16 family protein	6.42	24.10	6.07
MSMEG_2079	MSMEG_2079	alcohol dehydrogenase	6.39	6.75	6.56

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_4103	MSMEG_4103	alkanesulfonate monooxygenase	6.38	5.30	3.73
MSMEG_4645	orB	2-oxoglutarate ferredoxin oxidoreductase subunit beta	6.34	6.01	5.36
MSMEG_0793	thiG	thiazole synthase	6.29	7.50	6.31
MSMEG_5243	MSMEG_5243	helix-turn-helix motif	6.29	5.89	4.40
MSMEG_4527	MSMEG_4527	sulfite reductase [ferredoxin], EC Number - 1.8.7.1	6.28	6.51	6.28
MSMEG_2442	MSMEG_2442	ribonuclease HII, EC Number - 3.1.26.4	6.26	6.59	5.84
MSMEG_3086	tpiA	triosephosphate isomerase, EC Number - 5.3.1.1	6.24	7.10	4.90
MSMEG_4329	MSMEG_4329	propionyl-CoA carboxylase beta chain, EC Number - 6.4.1.3	6.23	6.31	4.76
MSMEG_3384	MSMEG_3384	isoflavone reductase	6.23	7.43	5.87
MSMEG_5530	MSMEG_5530	putative aliphatic sulfonates transport permease protein SsuC	6.20	6.06	5.51
MSMEG_0952	hemA	glutamyl-tRNA reductase, EC Number - 1.2.1.70	6.18	6.78	5.59
MSMEG_3861	MSMEG_3861	amidohydrolase 3	6.17	3.16	4.51
MSMEG_2918	MSMEG_2918	short-chain dehydrogenase,reductase SDR	6.14	5.22	4.95
MSMEG_0553	MSMEG_0553	probable sugar ABC transporter, substrate-binding protein, putative	6.13	5.81	6.17
MSMEG_4533	MSMEG_4533	sulfate-binding protein	6.12	5.51	5.55
MSMEG_2440	rplS	50S ribosomal protein L19	6.11	5.40	4.81
MSMEG_6523	MSMEG_6523	ABC transporter, membrane spanning protein	6.10	6.26	5.81
MSMEG_2841	MSMEG_2841	putative monooxygenase	6.09	5.01	4.29
MSMEG_0937	regX3	DNA-binding response regulator RegX3	6.06	6.92	5.95
MSMEG_5001	MSMEG_5001	pesticide degrading monooxygenase	6.04	5.30	5.28
MSMEG_2367	MSMEG_2367	aspartyl,glutamyl-tRNAAsn,Gln amidotransferase subunit B, EC Number - 6.3.5.-	6.03	5.60	6.07
MSMEG_6934	trx	thioredoxin	6.02	9.04	7.38
MSMEG_4891	MSMEG_4891	alkylhydroperoxide reductase	6.01	5.73	4.38
MSMEG_3259	MSMEG_3259	acyl-CoA dehydrogenase	6.00	13.00	6.03
MSMEG_0399	MSMEG_0399	hypothetical protein	6.00	5.31	5.08
MSMEG_2799	MSMEG_2799	phospho-2-dehydro-3-deoxyheptonate aldolase, EC Number - 2.5.1.54	5.99	5.99	5.93
MSMEG_1365	rplL	50S ribosomal protein L7,L12	5.97	6.19	5.08
MSMEG_3531	MSMEG_3531	hypothetical protein	5.96	5.32	5.36
MSMEG_1726	MSMEG_1726	transposase, similar to the proteins: MSMEG_4133	5.93	6.49	6.13
MSMEG_3053	rpoZ	DNA-directed RNA polymerase, omega subunit, EC Number - 2.7.7.6	5.92	5.71	5.19
MSMEG_2937	MSMEG_2937	pyridoxine biosynthesis protein	5.90	5.58	5.60
MSMEG_5248	MSMEG_5248	acyl-[ACP] desaturase	5.89	5.63	5.32
MSMEG_2723	recA	protein RecA	5.89	7.22	5.36
MSMEG_3512	MSMEG_3512	competence damage-inducible protein A	5.87	7.65	5.00
MSMEG_5048	MSMEG_5048	hypothetical protein	5.84	6.32	5.95
MSMEG_5244	MSMEG_5244	two component transcriptional regulatory protein devr	5.84	5.83	5.51
MSMEG_3327	MSMEG_3327	hypothetical protein	5.83	3.99	5.29
MSMEG_3640	glcB	malate synthase G, EC Number - 2.3.3.9	5.82	5.69	5.12
MSMEG_5122	MSMEG_5122	ferredoxin	5.76	6.39	4.97
MSMEG_4540	MSMEG_4540	hypothetical protein	5.73	4.85	4.42

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_3513	MSMEG_3513	hypothetical protein	5.72	5.00	4.79
MSMEG_2299	MSMEG_2299	ribonucleoside-diphosphate reductase, alpha subunit, EC Number - 1.17.4.1, similar to the proteins: MSMEG_1019	5.72	6.24	4.85
MSMEG_2752	MSMEG_2752	RNA polymerase sigma factor SigB	5.69	9.16	7.50
MSMEG_0394	MSMEG_0394	hypothetical protein	5.67	6.92	4.62
MSMEG_3793	infC	translation initiation factor IF-3	5.67	5.88	4.87
MSMEG_6933	trxB	thioredoxin-disulfide reductase, EC Number - 1.8.1.9	5.66	7.35	5.95
MSMEG_4543	MSMEG_4543	ABC transporter system integral membrane protein	5.64	5.42	4.05
MSMEG_2542	MSMEG_2542	integral membrane protein DUF6	5.62	5.36	6.02
MSMEG_2627	MSMEG_2627	hypothetical protein	5.56	4.39	5.43
MSMEG_6209	MSMEG_6209	hypothetical protein	5.55	5.91	4.33
MSMEG_3199	nadA	quinolinate synthetase	5.54	5.20	5.03
MSMEG_6671	MSMEG_6671	sulfate,thiosulfate import ATP-binding protein CysA, EC Number - 3.6.3.25	5.54	5.44	6.10
MSMEG_4529	MSMEG_4529	secreted protein	5.52	4.88	4.43
MSMEG_4935	atpC	ATP synthase F1, epsilon subunit, EC Number - 3.6.3.14	5.52	5.87	5.40
MSMEG_6858	MSMEG_6858	epoxide hydrolase 1, EC Number - 3.3.2.3	5.50	4.57	6.01
MSMEG_4669	fdhD	formate dehydrogenase accessory protein	5.48	5.04	5.00
MSMEG_0415	MSMEG_0415	NADH-fmn oxidoreductase	5.48	5.37	5.08
MSMEG_2695	MSMEG_2695	35 kDa protein	5.48	6.27	5.31
MSMEG_1340	MSMEG_1340	hypothetical protein	5.46	5.86	5.57
MSMEG_0558	MSMEG_0558	hypothetical protein	5.44	6.74	5.54
MSMEG_3276	MSMEG_3276	integral membrane protein	5.43	4.71	5.10
MSMEG_1540	MSMEG_1540	ATP-dependent RNA helicase	5.42	4.97	5.31
MSMEG_5770	MSMEG_5770	hypothetical protein	5.41	5.35	6.01
MSMEG_4539	MSMEG_4539	alkanesulfonate monooxygenase, EC Number - 1.14.14.5	5.36	7.91	4.88
MSMEG_3141	MSMEG_3141	hypothetical protein	5.36	6.06	4.31
MSMEG_4101	MSMEG_4101	ABC-type transporter, substrate binding protein	5.35	5.57	4.20
MSMEG_3619	MSMEG_3619	short chain dehydrogenase	5.32	5.50	4.37
MSMEG_0066	MSMEG_0066	early secretory antigenic target, 6 kDa	5.32	5.60	4.42
MSMEG_4286	lipA	lipoic acid synthetase, EC Number - 2.8.1.-	5.31	5.27	4.51
MSMEG_0688	MSMEG_0688	aminotransferase AlaT	5.30	5.93	4.59
MSMEG_1364	MSMEG_1364	50S ribosomal protein L10	5.30	5.31	4.36
MSMEG_2843	ssuD	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.-	5.28	4.00	2.99
MSMEG_3222	MSMEG_3222	prolipoprotein diacylglyceryl transferase, EC Number - 2.4.99.-	5.28	4.93	5.25
MSMEG_5788	MSMEG_5788	integral membrane protein	5.28	4.65	4.48
MSMEG_4917	MSMEG_4917	tetratricopeptide repeat domain protein	5.27	5.27	4.84
MSMEG_5558	MSMEG_5558	hypothetical protein	5.26	5.31	4.78
MSMEG_3526	MSMEG_3526	hypothetical protein	5.25	4.94	2.69
MSMEG_4216	MSMEG_4216	hypothetical protein	5.23	4.64	4.18
MSMEG_1604	MSMEG_1604	FAD dependent oxidoreductase	5.23	4.82	4.71

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_4673	clpP	Clp protease, EC Number - 3.4.21.92	5.22	6.11	4.39
MSMEG_5816	MSMEG_5816	hypothetical protein	5.21	9.87	4.86
MSMEG_0845	MSMEG_0845	putative monovalent cation,H+ antiporter subunit E	5.20	7.29	5.23
MSMEG_3681	MSMEG_3681	hypothetical protein	5.20	4.85	4.49
MSMEG_4623	MSMEG_4623	GTPase ObgE	5.19	4.69	4.97
MSMEG_3564	bfr	bacterioferritin	5.19	4.93	3.29
MSMEG_4548	MSMEG_4548	acetyltransferase, gnat family protein	5.18	4.82	4.01
MSMEG_0647	phnC	phosphonate ABC transporter, ATP-binding protein, EC Number - 3.6.3.28	5.18	9.11	5.53
MSMEG_3661	MSMEG_3661	hypothetical protein	5.17	5.29	3.82
MSMEG_6248	MSMEG_6248	hypothetical protein	5.16	5.44	5.68
MSMEG_1602	guaB	inosine-5-monophosphate dehydrogenase, EC Number - 1.1.1.205	5.16	5.20	4.68
MSMEG_1474	rplO	50S ribosomal protein L15	5.15	5.78	5.71
MSMEG_6783	MSMEG_6783	integral membrane protein	5.15	5.40	4.42
MSMEG_5733	MSMEG_5733	universal stress protein family protein	5.12	4.94	4.09
MSMEG_2694	MSMEG_2694	transcriptional regulator, XRE family protein	5.12	5.45	5.02
MSMEG_3871	cobG	precorrin-3B synthase, EC Number - 1.14.13.83	5.11	4.26	4.37
MSMEG_5757	MSMEG_5757	Glu tRNA	5.11	5.23	3.75
MSMEG_4674	tig	trigger factor, EC Number - 5.2.1.8	5.11	4.75	4.61
MSMEG_3084	gap	glyceraldehyde-3-phosphate dehydrogenase, type I, EC Number - 1.2.1.12	5.09	4.88	4.09
MSMEG_3031	MSMEG_3031	shikimate kinase, EC Number - 2.7.1.71	5.09	4.65	4.72
MSMEG_4309	MSMEG_4309	Low molecular weight protein-tyrosine-phosphatase, EC Number - 3.1.3.48	5.08	4.49	5.25
MSMEG_1243	MSMEG_1243	hypothetical protein	5.06	5.83	4.12
MSMEG_2437	rimM	16S rRNA-processing protein	5.06	4.80	4.46
MSMEG_4214	MSMEG_4214	glutamine amidotransferase	5.05	4.38	3.89
MSMEG_4954	rho	transcription termination factor Rho	5.03	5.50	5.41
MSMEG_3143	acnA	aconitate hydratase 1, EC Number - 4.2.1.3	5.00	11.95	4.07
MSMEG_1344	MSMEG_1344	preprotein translocase subunit SecE	4.96	5.83	4.80
MSMEG_5084	MSMEG_5084	glycosyl transferase, group 2 family protein, EC Number - 2.4.1.-	4.95	4.83	4.70
MSMEG_6078	MSMEG_6078	LpqE protein	4.94	4.47	4.38
MSMEG_3895	MSMEG_3895	proteasome beta subunit	4.94	4.86	4.16
MSMEG_5049	sucA	2-oxoglutarate dehydrogenase, E1 component, EC Number - 1.2.4.2	4.91	7.84	4.83
MSMEG_3580	MSMEG_3580	antigen 85-C, EC Number - 2.3.1.-	4.90	4.95	4.20
MSMEG_1597	MSMEG_1597	Transcription factor WhiB	4.90	4.10	3.90
MSMEG_3631	MSMEG_3631	integral membrane protein	4.89	4.42	4.46
MSMEG_6398	MSMEG_6398	antigen 85-A, EC Number - 2.3.1.-	4.89	5.75	5.09
MSMEG_0679	MSMEG_0679	hypothetical protein	4.88	4.58	4.85
MSMEG_4095	MSMEG_4095	putative monooxygenase	4.87	4.18	2.90
MSMEG_4559	MSMEG_4559	ABC transporter, membrane spanning protein	4.87	4.28	3.96
MSMEG_4325	MSMEG_4325	malonyl CoA-acyl carrier protein transacylase, EC Number - 2.3.1.39	4.86	4.84	3.96
MSMEG_4400	MSMEG_4400	alcohol dehydrogenase, zinc-containing	4.85	4.37	4.96

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_0933	MSMEG_0933	hypothetical protein	4.85	4.17	4.69
MSMEG_4495	MSMEG_4495	CBS domain protein	4.85	4.85	3.66
MSMEG_1342	MSMEG_1342	hypothetical protein	4.84	5.25	4.79
MSMEG_3834	MSMEG_3834	putative TetR-family protein transcriptional regulator	4.83	5.91	4.71
MSMEG_4757	MSMEG_4757	fatty acid synthase	4.83	4.64	4.95
MSMEG_2776	dxs	1-deoxy-D-xylulose-5-phosphate synthase, EC Number - 2.2.1.7	4.82	3.79	3.92
MSMEG_2758	MSMEG_2758	RNA polymerase sigma factor RpoD	4.81	5.69	4.67
MSMEG_1339	rpmG	ribosomal protein L33	4.81	4.90	4.66
MSMEG_4340	MSMEG_4340	NAD,mycothiol-dependent formaldehyde dehydrogenase, EC Number - 1.2.1.66	4.76	4.67	3.83
MSMEG_4542	MSMEG_4542	oligopeptide transport integral membrane protein	4.76	9.63	4.28
MSMEG_3066	rpe	ribulose-phosphate 3-epimerase, EC Number - 5.1.3.1	4.75	4.55	4.30
MSMEG_3127	MSMEG_3127	conserved protein, DUF59	4.73	4.79	4.52
MSMEG_3122	sufB	FeS assembly protein SufB	4.70	9.49	4.52
MSMEG_4100	MSMEG_4100	ABC transporter permease protein	4.70	16.73	3.97
MSMEG_2622	MSMEG_2622	conserved hypothetical alanine rich protein	4.69	3.78	4.19
MSMEG_1813	MSMEG_1813	propionyl-CoA carboxylase beta chain, EC Number - 6.4.1.3	4.68	4.68	3.65
MSMEG_4344	MSMEG_4344	putative monooxygenase	4.68	4.37	4.80
MSMEG_4644	MSMEG_4644	molybdopterin-guanine dinucleotide biosynthesis protein A	4.66	4.93	3.89
MSMEG_5680	MSMEG_5680	glyoxalase family protein	4.65	4.34	3.25
MSMEG_2743	nrdR	hypothetical protein	4.62	4.89	3.95
MSMEG_2735	dapF	diaminopimelate epimerase, EC Number - 5.1.1.7	4.61	3.87	4.34
MSMEG_2400	rpmB	50S ribosomal protein L28	4.60	4.72	4.56
MSMEG_3178	MSMEG_3178	DNA polymerase III alpha subunit, EC Number - 2.7.7.7	4.60	4.59	4.21
MSMEG_5672	gltA	citrate synthase I, EC Number - 4.1.3.7	4.60	4.56	4.68
MSMEG_2938	tesB	acyl-CoA thioesterase II, EC Number - 3.1.2.-	4.60	4.04	3.96
MSMEG_1843	ahcY	adenosylhomocysteinase, EC Number - 3.3.1.1	4.59	4.54	4.32
MSMEG_4217	MSMEG_4217	DivIVA protein	4.58	4.08	4.04
MSMEG_1622	MSMEG_1622	putative DNA repair polymerase	4.58	4.24	4.29
MSMEG_2768	MSMEG_2768	OB-fold nucleic acid binding domain protein	4.58	5.75	4.44
MSMEG_3758	tyrS	tyrosyl-tRNA synthetase, EC Number - 6.1.1.1	4.57	4.05	3.80
MSMEG_5889	MSMEG_5889	hypothetical protein	4.57	4.05	4.24
MSMEG_3052	MSMEG_3052	hypothetical protein	4.56	4.84	3.56
MSMEG_4907	MSMEG_4907	Mov34,MPN,PAD-1 family protein	4.55	5.31	4.55
MSMEG_1322	MSMEG_1322	ErfK,YbiS,YcfS,YnhG family protein	4.54	4.95	3.87
MSMEG_5293	MSMEG_5293	hypothetical protein	4.54	4.81	4.46
MSMEG_5121	MSMEG_5121	N-succinyldiaminopimelate aminotransferase	4.52	4.37	4.51
MSMEG_3935	MSMEG_3935	hypothetical protein	4.51	4.67	3.12
MSMEG_3044	MSMEG_3044	dihydroorotase, EC Number - 3.5.2.3	4.51	4.19	4.42
MSMEG_3073	ribE	6,7-dimethyl-8-ribityllumazine synthase, EC Number - 6.3.3.-	4.51	4.04	3.86
MSMEG_6076	ispD	2-C-methyl-D-erythritol 4-phosphate	4.51	4.21	4.54

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
		cytidylyltransferase, EC Number - 2.7.7.60			
MSMEG_5534	pcrA	ATP-dependent DNA helicase PcrA, EC Number - 3.6.1.-	4.49	4.65	4.42
MSMEG_1272	MSMEG_1272	putative ribosylglycohydrolase	4.48	7.36	4.45
MSMEG_3858	MSMEG_3858	hypothetical protein	4.46	4.14	3.51
MSMEG_2730	MSMEG_2730	tRNA-I6A37 thiotransferase enzyme MiaB	4.46	4.13	3.74
MSMEG_2436	MSMEG_2436	hypothetical protein	4.45	4.46	4.12
MSMEG_4287	MSMEG_4287	integral membrane protein	4.45	4.53	4.23
MSMEG_2093	MSMEG_2093	sRNA	4.45	5.28	5.30
MSMEG_0969	hemL	glutamate-1-semialdehyde-2,1-aminomutase, EC Number - 5.4.3.8	4.43	4.17	4.32
MSMEG_2401	MSMEG_2401	hypothetical protein	4.42	4.22	4.10
MSMEG_3081	MSMEG_3081	hypothetical protein	4.42	3.69	3.84
MSMEG_4492	MSMEG_4492	amidase	4.41	3.98	3.93
MSMEG_3210	MSMEG_3210	inositol monophosphate phosphatase	4.40	4.45	3.93
MSMEG_3219	trpC	indole-3-glycerol phosphate synthase, EC Number - 4.1.1.48	4.40	4.13	4.17
MSMEG_1671	MSMEG_1671	succinate dehydrogenase hydrophobic membrane anchor protein SdhD	4.40	6.90	4.17
MSMEG_5612	MSMEG_5612	amino-acid acetyltransferase	4.39	3.32	3.73
MSMEG_0943	proC	pyrroline-5-carboxylate reductase, EC Number - 1.5.1.2	4.39	4.91	4.05
MSMEG_6082	MSMEG_6082	carbonic anhydrase	4.38	4.97	4.34
MSMEG_1028	MSMEG_1028	geranylgeranyl reductase, similar to the proteins: MSMEG_2308	4.38	4.41	4.55
MSMEG_6896	MSMEG_6896	single-strand DNA-binding protein	4.37	5.00	4.88
MSMEG_2975	MSMEG_2975	metallo-beta-lactamase family protein	4.37	4.24	4.11
MSMEG_4668	MSMEG_4668	oxidoreductase alpha molybdopterin subunit	4.36	3.70	4.20
MSMEG_3620	MSMEG_3620	hypothetical protein	4.36	3.90	3.50
MSMEG_3225	MSMEG_3225	ferredoxin-dependent glutamate synthase 1, EC Number - 1.4.7.1	4.35	4.30	4.15
MSMEG_4221	MSMEG_4221	hypothetical protein	4.35	4.13	4.03
MSMEG_1392	MSMEG_1392	alcohol dehydrogenase, class IV	4.34	4.24	4.36
MSMEG_5831	purQ	phosphoribosylformylglycinamidine synthase I, EC Number - 6.3.5.3	4.33	4.50	4.27
MSMEG_2541	frr	ribosome recycling factor	4.33	4.72	4.72
MSMEG_1601	MSMEG_1601	hypothetical protein	4.30	4.03	3.48
MSMEG_5700	MSMEG_5700	secreted protein	4.29	3.25	3.72
MSMEG_3099	pgl	6-phosphogluconolactonase, EC Number - 3.1.1.31	4.29	3.88	4.01
MSMEG_3025	alaS	alanyl-tRNA synthetase, EC Number - 6.1.1.7	4.29	3.61	3.94
MSMEG_5247	MSMEG_5247	PhoH family protein	4.29	4.05	3.81
MSMEG_2834	MSMEG_2834	Cys tRNA	4.29	3.74	3.67
MSMEG_4671	clpX	ATP-dependent protease ATP-binding subunit	4.28	4.15	3.84
MSMEG_0954	hemD	uroporphyrinogen-III synthase, EC Number - 4.2.1.75	4.27	4.24	4.44
MSMEG_3140	MSMEG_3140	ABC transporter ATP-binding protein, possibly in EF-3 subfamily protein	4.27	4.01	4.11
MSMEG_3952	MSMEG_3952	hypothetical protein	4.25	4.37	3.27
MSMEG_5102	MSMEG_5102	ABC transporter ATP-binding protein	4.23	4.14	4.24

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_3854	MSMEG_3854	putative aliphatic sulfonates transport permease protein SsuC	4.23	3.68	3.76
MSMEG_4102	MSMEG_4102	hypothetical protein	4.22	7.02	2.96
MSMEG_6271	leuA	2-isopropylmalate synthase, EC Number - 2.3.3.13	4.20	4.80	4.63
MSMEG_1947	MSMEG_1947	hypothetical protein	4.19	9.84	5.09
MSMEG_0414	MSMEG_0414	oxidoreductase, 2OG-FeII oxygenase family protein	4.19	4.20	4.16
MSMEG_3527	MSMEG_3527	putative HTH-type transcriptional regulator	4.19	4.11	3.52
MSMEG_1654	MSMEG_1654	isocitrate dehydrogenase, NADP-dependent, EC Number - 1.1.1.42	4.19	4.01	3.89
MSMEG_1914	MSMEG_1914	RNA polymerase sigma-70 factor, family protein	4.18	8.73	5.48
MSMEG_2724	MSMEG_2724	recombination regulator RecX	4.18	3.98	3.56
MSMEG_2625	nusA	transcription elongation factor NusA	4.17	3.90	3.92
MSMEG_6414	MSMEG_6414	hypothetical protein	4.13	3.45	4.11
MSMEG_5292	MSMEG_5292	hypothetical protein	4.12	5.33	4.27
MSMEG_1033	MSMEG_1033	ribonucleoside-diphosphate reductase, beta subunit, EC Number - 1.17.4.1, similar to the proteins: MSMEG_2313	4.11	5.80	3.73
MSMEG_3208	hisH	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit, EC Number - 2.4.2.-	4.11	3.86	3.72
MSMEG_2188	MSMEG_2188	integral membrane protein	4.11	4.13	3.84
MSMEG_3752	MSMEG_3752	hypothetical protein	4.11	3.74	3.37
MSMEG_4672	clpP	Clp protease, EC Number - 3.4.21.92	4.10	4.13	3.93
MSMEG_0233	MSMEG_0233	lipoprotein Lpps	4.09	3.91	3.90
MSMEG_2962	MSMEG_2962	preprotein translocase subunit SecF	4.08	3.35	3.21
MSMEG_3713	MSMEG_3713	hypothetical protein	4.08	3.67	3.60
MSMEG_1403	MSMEG_1403	cutinase superfamily protein	4.08	3.97	4.57
MSMEG_2378	serA	D-3-phosphoglycerate dehydrogenase, EC Number - 1.1.1.95	4.07	3.85	3.81
MSMEG_3072	MSMEG_3072	riboflavin biosynthesis protein ribAB, EC Number - 3.5.4.25	4.07	4.23	4.09
MSMEG_2357	MSMEG_2357	cysteine desulfurase, EC Number - 2.8.1.7	4.07	3.74	4.11
MSMEG_6090	MSMEG_6090	hypothetical protein	4.06	4.79	3.87
MSMEG_0919	MSMEG_0919	heparin-binding hemagglutinin	4.06	4.12	4.13
MSMEG_0422	MSMEG_0422	transferase	4.05	4.03	4.39
MSMEG_3209	MSMEG_3209	1-5-phosphoribosyl-5-[5-phosphoribosylaminomethylideneamino] imidazole-4-carboxamide isomerase	4.03	2.58	3.67
MSMEG_1165	MSMEG_1165	nucleotide-binding protein	4.03	4.29	4.08
MSMEG_6202	MSMEG_6202	secreted protein	4.02	4.52	4.12
MSMEG_3026	MSMEG_3026	Holliday junction resolvase-like protein	4.02	3.51	3.33
MSMEG_5810	MSMEG_5810	putative monooxygenase	4.01	3.68	4.00
MSMEG_3852	MSMEG_3852	aliphatic sulfonate binding protein	4.01	3.54	3.82
MSMEG_3030	aroC	chorismate synthase, EC Number - 4.2.3.5	4.01	3.59	4.01
MSMEG_1041	serB	phosphoserine phosphatase, EC Number - 3.1.3.3, similar to the proteins: MSMEG_2321	4.01	3.46	3.75
MSMEG_2374	ilvC	ketol-acid reductoisomerase, EC Number - 1.1.1.86	4.01	10.22	3.79
MSMEG_0449	MSMEG_0449	transporter, major facilitator family protein, putative	3.99	3.84	4.16
MSMEG_5223	MSMEG_5223	hypothetical protein	3.99	3.48	3.48

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_4240	MSMEG_4240	polyprenyl synthetase	3.98	3.67	3.90
MSMEG_4646	MSMEG_4646	pyruvate synthase	3.98	3.94	3.93
MSMEG_3670	MSMEG_3670	transporter, small multidrug resistance SMR family protein	3.97	3.71	3.61
MSMEG_4092	MSMEG_4092	alkanal monooxygenase	3.97	3.58	2.60
MSMEG_5047	MSMEG_5047	drug resistance transporter Bcr,CflA subfamily protein	3.97	10.47	5.23
MSMEG_4905	cysM	cysteine synthase B, EC Number - 2.5.1.47	3.97	4.27	4.02
MSMEG_0931	MSMEG_0931	hypothetical protein	3.94	3.89	3.43
MSMEG_2688	MSMEG_2688	antibiotic biosynthesis monooxygenase domain protein	3.93	3.56	3.34
MSMEG_3221	trpA	tryptophan synthase, alpha subunit, EC Number - 4.2.1.20	3.92	3.21	3.34
MSMEG_1881	secA	preprotein translocase subunit SecA	3.91	3.68	3.58
MSMEG_1515	MSMEG_1515	two-component sensor histidine kinase	3.91	4.19	3.88
MSMEG_5239	glpX	fructose-1,6-bisphosphatase, class II, EC Number - 3.1.3.11	3.90	3.83	3.65
MSMEG_6201	MSMEG_6201	Transglycosylase	3.89	3.79	4.00
MSMEG_4977	MSMEG_4977	3'2',5'-bisphosphate nucleotidase, putative	3.89	3.08	3.83
MSMEG_2621	proS	prolyl-tRNA synthetase, EC Number - 6.1.1.15	3.88	3.42	3.93
MSMEG_0834	MSMEG_0834	tuberculin related peptide	3.88	3.96	4.38
MSMEG_3207	hisB	imidazoleglycerol-phosphate dehydratase, EC Number - 4.2.1.19	3.87	3.89	3.91
MSMEG_1271	MSMEG_1271	hypothetical protein	3.84	3.83	3.42
MSMEG_2177	MSMEG_2177	fmnh2-utilizing oxygenase	3.84	3.98	3.70
MSMEG_4263	MSMEG_4263	ubiquinol-cytochrome c reductase cytochrome b subunit	3.84	3.84	3.60
MSMEG_3644	MSMEG_3644	transcriptional regulator, MerR family protein	3.84	4.10	3.40
MSMEG_0643	MSMEG_0643	extracellular solute-binding protein, family protein 5, putative	3.82	4.58	3.81
MSMEG_4544	MSMEG_4544	methyltransferase small domain superfamily protein	3.82	9.19	3.58
MSMEG_2765	MSMEG_2765	deoxyuridine 5-triphosphate nucleotidohydrolase, EC Number - 3.6.1.23	3.80	2.98	3.23
MSMEG_3772	argF	ornithine carbamoyltransferase, EC Number - 2.1.3.3	3.79	3.12	3.06
MSMEG_4896	MSMEG_4896	acyl-CoA synthetase	3.79	4.33	4.07
MSMEG_5042	MSMEG_5042	ATP-dependent rna helicase, dead,deah box family protein	3.77	3.56	3.08
MSMEG_1821	MSMEG_1821	acyl-CoA dehydrogenase, EC Number - 1.3.99.-	3.77	4.52	3.54
MSMEG_0866	MSMEG_0866	DNA or RNA helicase of superfamily protein II	3.75	3.94	3.80
MSMEG_4621	proB	glutamate 5-kinase, EC Number - 2.7.2.11	3.74	3.34	3.35
MSMEG_1113	MSMEG_1113	glycosyl transferase	3.74	3.40	3.99
MSMEG_2833	MSMEG_2833	Val tRNA	3.73	3.90	3.47
MSMEG_2684	dapA	dihydrodipicolinate synthase, EC Number - 4.2.1.52	3.72	3.14	3.55
MSMEG_1026	MSMEG_1026	putative acetyltransferase, similar to the proteins: MSMEG_2306	3.71	3.69	3.98
MSMEG_4690	pepN	aminopeptidase N, EC Number - 3.4.11.2	3.71	3.35	3.25
MSMEG_6105	MSMEG_6105	cell division protein	3.71	3.60	4.14
MSMEG_6946	rpmH	50S ribosomal protein L34	3.70	3.64	4.24

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_0987	MSMEG_0987	hypothetical protein	3.69	3.91	3.72
MSMEG_3872	MSMEG_3872	precorrin-8X methylmutase, EC Number - 5.4.1.2	3.68	3.45	3.27
MSMEG_3907	MSMEG_3907	RecB family protein exonuclease	3.67	3.31	3.27
MSMEG_4218	MSMEG_4218	possible conserved transmembrane protein	3.67	3.99	3.53
MSMEG_1819	purK	phosphoribosylaminoimidazole carboxylase, ATPase subunit, EC Number - 4.1.1.21	3.67	3.75	4.44
MSMEG_4700	MSMEG_4700	putative ABC transporter ATP-binding protein	3.66	3.45	3.59
MSMEG_3747	MSMEG_3747	CTP synthetase	3.66	3.41	3.22
MSMEG_0769	metZ	O-succinylhomoserine sulfhydrylase, EC Number - 4.2.99.-	3.65	3.53	3.36
MSMEG_4283	sucB	2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase, EC Number - 2.3.1.61	3.65	13.63	3.50
MSMEG_3051	MSMEG_3051	guanylate kinase, EC Number - 2.7.4.8	3.65	2.89	3.05
MSMEG_3101	zwf	glucose-6-phosphate 1-dehydrogenase, EC Number - 1.1.1.49	3.64	3.74	3.32
MSMEG_1929	MSMEG_1929	hypothetical protein	3.64	4.31	3.41
MSMEG_3058	MSMEG_3058	lipoprotein, nlpa family protein	3.64	4.41	3.82
MSMEG_4951	rpmE	ribosomal protein L31	3.63	4.38	3.67
MSMEG_0680	MSMEG_0680	UDP-glucose 6-dehydrogenase, EC Number - 1.1.1.22	3.62	3.09	3.89
MSMEG_4284	MSMEG_4284	hypothetical protein	3.62	3.72	3.37
MSMEG_5196	MSMEG_5196	fasciclin domain protein	3.62	3.45	3.30
MSMEG_3748	MSMEG_3748	thiamin pyrophosphokinase, catalytic domain protein	3.61	3.67	3.48
MSMEG_5136	MSMEG_5136	helix-turn-helix motif	3.61	3.57	2.67
MSMEG_6104	folE	GTP cyclohydrolase I, EC Number - 3.5.4.16	3.60	3.58	3.60
MSMEG_3183	ilvA	threonine dehydratase, EC Number - 4.3.1.19	3.59	3.43	3.48
MSMEG_2840	MSMEG_2840	hypothetical protein	3.59	2.91	2.77
MSMEG_3632	gnd	6-phosphogluconate dehydrogenase, decarboxylating, EC Number - 1.1.1.44	3.58	3.30	3.19
MSMEG_6025	MSMEG_6025	hypothetical protein	3.57	3.54	3.16
MSMEG_2615	MSMEG_2615	chelatase	3.57	3.67	3.44
MSMEG_2416	MSMEG_2416	hypothetical protein	3.56	4.17	3.81
MSMEG_3945	MSMEG_3945	universal stress protein family protein	3.56	3.08	2.44
MSMEG_3227	pyk	pyruvate kinase, EC Number - 2.7.1.40	3.55	3.61	3.27
MSMEG_0010	MSMEG_0010	Ala tRNA	3.54	3.64	2.99
MSMEG_2412	pyc	pyruvate carboxylase, EC Number - 6.4.1.1	3.54	3.07	3.51
MSMEG_4237	MSMEG_4237	probable conserved transmembrane protein	3.53	3.23	2.95
MSMEG_0847	MSMEG_0847	putative monovalent cation,H+ antiporter subunit C	3.53	3.69	3.45
MSMEG_6402	MSMEG_6402	PAP2 superfamily protein	3.52	3.62	3.41
MSMEG_5269	MSMEG_5269	hypothetical protein	3.52	3.46	3.33
MSMEG_4978	cysNC	sulfate adenylytransferase, large subunit,adenylylsulfate kinase, EC Number - 2.7.7.4 2.7.1.26	3.51	3.71	3.63
MSMEG_4385	MSMEG_4385	ABC transporter oligopeptide binding protein	3.50	3.28	2.76
MSMEG_3942	MSMEG_3942	hypothetical protein	3.50	3.39	3.13
MSMEG_5679	MSMEG_5679	putative DNA-binding protein	3.50	3.43	2.83

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_5773	MSMEG_5773	fatty acid desaturase	3.49	3.45	3.28
MSMEG_5890	MSMEG_5890	hypothetical protein	3.49	4.07	3.67
MSMEG_3808	uvrA	excinuclease ABC subunit A	3.48	3.90	3.46
MSMEG_0792	thiS	sulfur carrier protein ThiS	3.46	3.09	3.78
MSMEG_5072	MSMEG_5072	RNA polymerase sigma factor SigE	3.45	3.56	2.39
MSMEG_3087	secG	preprotein translocase subunit SecG	3.43	3.00	3.07
MSMEG_3211	hisF	imidazole glycerol phosphate synthase subunit HisF	3.43	2.98	3.33
MSMEG_5249	glyA	serine hydroxymethyltransferase, EC Number - 2.1.2.1	3.43	3.37	3.33
MSMEG_1575	alr	alanine racemase, EC Number - 5.1.1.1	3.42	3.16	3.42
MSMEG_5004	MSMEG_5004	DNA repair exonuclease	3.41	3.16	3.28
MSMEG_1680	MSMEG_1680	hypothetical protein	3.40	3.32	2.30
MSMEG_2080	MSMEG_2080	putative acyl-CoA dehydrogenase	3.40	3.23	3.35
MSMEG_3139	MSMEG_3139	enoyl-CoA hydratase	3.39	3.78	3.23
MSMEG_2373	ilvN	acetolactate synthase, small subunit, EC Number - 2.2.1.6	3.39	2.88	2.44
MSMEG_6544	MSMEG_6544	transport-associated, putative	3.38	116.97	3.35
MSMEG_2782	MSMEG_2782	hypothetical protein	3.38	3.57	3.53
MSMEG_4950	prfA	peptide chain release factor 1	3.38	3.00	3.36
MSMEG_0693	MSMEG_0693	hypothetical protein	3.37	3.46	2.77
MSMEG_5685	MSMEG_5685	DNA-binding protein	3.37	3.08	3.37
MSMEG_0288	MSMEG_0288	FAD dependent oxidoreductase, putative	3.37	3.45	3.34
MSMEG_2418	rnc	ribonuclease III, EC Number - 3.1.26.3	3.36	2.85	2.88
MSMEG_3853	MSMEG_3853	putative aliphatic sulfonates transport ATP-binding protein SsuB	3.35	2.81	3.22
MSMEG_1188	MSMEG_1188	hypothetical protein	3.34	13.32	3.36
MSMEG_5423	mfd	transcription-repair coupling factor	3.34	5.37	3.38
MSMEG_2750	MSMEG_2750	iron-dependent repressor IdeR	3.33	4.34	3.16
MSMEG_5375	MSMEG_5375	GntR-family protein transcriptional regulator	3.31	3.56	3.17
MSMEG_2650	MSMEG_2650	acyl-CoA dehydrogenase	3.31	3.18	3.45
MSMEG_4235	mraW	S-adenosyl-methyltransferase MraW, EC Number - 2.1.1.-	3.31	3.05	3.22
MSMEG_4244	MSMEG_4244	3-deoxy-7-phosphoheptulonate synthase, EC Number - 2.5.1.54	3.30	3.10	3.56
MSMEG_4979	cysD	sulfate adenylyltransferase, small subunit, EC Number - 2.7.7.4	3.30	2.89	3.18
MSMEG_2527	MSMEG_2527	hypothetical protein	3.29	2.92	3.18
MSMEG_3220	trpB	tryptophan synthase, beta subunit, EC Number - 4.2.1.20	3.29	2.99	3.06
MSMEG_1647	MSMEG_1647	tetrahydrofolate dehydrogenase,cyclohydrolase FolD	3.28	2.78	3.11
MSMEG_4096	ssuD	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.-	3.27	2.84	2.52
MSMEG_3185	MSMEG_3185	putative maltooligosyl trehalose synthase, EC Number - 5.4.99.15	3.27	2.45	2.41
MSMEG_6565	MSMEG_6565	hypothetical protein	3.27	3.63	3.93
MSMEG_4234	MSMEG_4234	hypothetical protein	3.26	3.50	3.01
MSMEG_4496	MSMEG_4496	hypothetical protein	3.25	3.11	3.28

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_2084	MSMEG_2084	hypothetical protein	3.25	3.16	2.98
MSMEG_0401	MSMEG_0401	putative non-ribosomal peptide synthase	3.23	4.93	3.30
MSMEG_4921	mce	methylmalonyl-CoA epimerase, EC Number - 5.1.99.1	3.23	3.21	2.94
MSMEG_1042	MSMEG_1042	hypothetical protein, similar to the proteins: MSMEG_2322	3.23	3.11	3.38
MSMEG_3477	MSMEG_3477	possible inv protein	3.22	2.82	3.02
MSMEG_2757	MSMEG_2757	glutathione S-transferase domain protein	3.22	3.49	3.20
MSMEG_1760	MSMEG_1760	short-chain dehydrogenase,reductase SDR	3.22	8.63	3.61
MSMEG_3186	glgX	glycogen debranching enzyme GlgX, EC Number - 3.2.1.-	3.21	3.13	2.81
MSMEG_4176	MSMEG_4176	hypothetical protein	3.20	3.12	3.06
MSMEG_2647	MSMEG_2647	metallophosphoesterase	3.20	3.12	2.64
MSMEG_6385	MSMEG_6385	hypothetical oxidoreductase in MprA 5region, EC Number - 1.-.-.-	3.19	2.83	3.13
MSMEG_0113	MSMEG_0113	taurine transport system permease protein TauC	3.19	7.30	3.81
MSMEG_4537	MSMEG_4537	major membrane protein I	3.19	3.64	3.03
MSMEG_0710	grpE	co-chaperone GrpE	3.19	4.85	4.21
MSMEG_1642	MSMEG_1642	ABC transporter, ATP-binding protein	3.18	3.13	3.07
MSMEG_1937	MSMEG_1937	molybdopterin biosynthesis-like protein MoeZ	3.18	3.51	3.26
MSMEG_6941	MSMEG_6941	R3H domain-containing protein	3.17	2.99	3.25
MSMEG_6089	MSMEG_6089	hypothetical protein	3.17	3.30	3.37
MSMEG_2424	ftsY	signal recognition particle-docking protein FtsY	3.17	3.05	3.26
MSMEG_4960	MSMEG_4960	Arg tRNA	3.17	2.73	2.80
MSMEG_5284	MSMEG_5284	patatin	3.16	2.65	3.02
MSMEG_5634	MSMEG_5634	hypothetical protein	3.16	3.64	3.38
MSMEG_4745	MSMEG_4745	ErfK,YbiS,YcfS,YnhG family protein	3.15	7.28	2.95
MSMEG_6729	MSMEG_6729	DNA-binding protein	3.13	3.35	3.15
MSMEG_1247	MSMEG_1247	hypothetical protein	3.12	3.34	2.71
MSMEG_4505	hrcA	heat-inducible transcription repressor	3.12	3.33	3.36
MSMEG_2729	miaB	tRNA-I6A37 thiotransferase enzyme MiaB	3.12	2.82	2.80
MSMEG_4504	dnaJ	chaperone protein DnaJ	3.12	2.77	2.77
MSMEG_2751	MSMEG_2751	hypothetical protein	3.11	3.36	3.26
MSMEG_3047	carB	carbamoyl-phosphate synthase, large subunit, EC Number - 6.3.5.5	3.11	2.95	2.91
MSMEG_0439	MSMEG_0439	hypothetical protein	3.10	3.12	3.25
MSMEG_0400	MSMEG_0400	peptide synthetase	3.10	2.89	3.08
MSMEG_3048	pyrF	orotidine 5-phosphate decarboxylase, EC Number - 4.1.1.23	3.09	2.72	3.08
MSMEG_4900	MSMEG_4900	Pks14 protein	3.09	3.24	2.73
MSMEG_3616	MSMEG_3616	integral membrane protein	3.08	3.13	2.84
MSMEG_4532	cysT	sulfate ABC transporter, permease protein CysT	3.08	3.02	3.13
MSMEG_3074	MSMEG_3074	hypothetical protein	3.08	3.08	3.04
MSMEG_3725	pqqA	coenzyme PQQ biosynthesis protein A	3.08	3.49	3.38
MSMEG_6487	MSMEG_6487	2-hydroxy-3-carboxy-6-oxo-7-methylocta-2, 4-dienoate decarboxylase	3.07	2.54	2.65
MSMEG_3232	cydB	cytochrome D ubiquinol oxidase, subunit II, EC Number - 1.10.3.-	3.07	3.15	2.46

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_3103	tkt	transketolase, EC Number - 2.2.1.1	3.06	3.04	2.78
MSMEG_1576	MSMEG_1576	alpha,beta hydrolase fold	3.06	2.99	2.91
MSMEG_5260	MSMEG_5260	hypothetical protein	3.05	3.18	3.40
MSMEG_2078	MSMEG_2078	antigen 85-C, EC Number - 2.3.1.-	3.05	3.07	3.00
MSMEG_4747	MSMEG_4747	hypothetical protein	3.05	3.02	2.84
MSMEG_1051	MSMEG_1051	immunogenic protein MPB64,MPT64, similar to the proteins: MSMEG_2331	3.05	2.44	2.19
MSMEG_2578	dxr	1-deoxy-D-xylulose 5-phosphate reductoisomerase, EC Number - 1.1.1.267	3.05	2.98	2.96
MSMEG_2397	MSMEG_2397	transcriptional regulatory protein, AsnC family protein	3.04	2.78	2.72
MSMEG_2417	MSMEG_2417	hypothetical protein	3.04	2.82	3.08
MSMEG_3816	uvrB	excinuclease ABC subunit B	3.04	3.00	2.90
MSMEG_1823	MSMEG_1823	hypothetical protein	3.03	3.14	3.58
MSMEG_3212	hisIE	phosphoribosyl-AMP pyrophosphatase,phosphoribosyl-ATP cyclohydrolase	3.03	2.96	2.56
MSMEG_3217	trpE	anthranilate synthase component I, EC Number - 4.1.3.27	3.03	2.71	2.90
MSMEG_2382	MSMEG_2382	5-carboxymethyl-2-hydroxymuconate delta-isomerase	3.03	3.24	2.82
MSMEG_3278	MSMEG_3278	hypothetical protein	3.03	2.69	2.71
MSMEG_3775	argJ	arginine biosynthesis bifunctional protein ArgJ, EC Number - 2.3.1.35 2.3.1.1	3.03	2.57	2.83
MSMEG_3688	MSMEG_3688	hypothetical protein	3.03	2.90	3.28
MSMEG_6725	MSMEG_6725	ABC transporter, ATP-binding protein	3.03	2.77	2.95
MSMEG_3004	MSMEG_3004	conserved hypothetical protein TIGR00026	3.02	3.02	2.69
MSMEG_1883	MSMEG_1883	glycine betaine transporter OpuD	3.02	2.75	2.88
MSMEG_0554	MSMEG_0554	ABC transporter permease protein, putative	3.02	3.31	3.24
MSMEG_4620	MSMEG_4620	NAD-dependent deacetylase 1, EC Number - 3.5.1.-	3.01	2.70	2.78
MSMEG_0702	MSMEG_0702	monooxygenase	3.01	2.82	2.85
MSMEG_2081	MSMEG_2081	putative acyl-CoA dehydrogenase	3.01	2.79	2.85
MSMEG_5241	MSMEG_5241	GAF family protein	3.00	2.79	2.87
MSMEG_0776	MSMEG_0776	hypothetical protein	3.00	3.34	3.00
MSMEG_5151	MSMEG_5151	hypothetical protein	2.99	2.29	2.70
MSMEG_3244	MSMEG_3244	hypothetical protein	2.99	2.67	2.96
MSMEG_1270	MSMEG_1270	hypothetical protein	2.99	16.03	3.05
MSMEG_1610	MSMEG_1610	GMP synthase [glutamine-hydrolyzing], EC Number - 6.3.5.2	2.99	2.84	2.90
MSMEG_0848	MSMEG_0848	putative monovalent cation,H+ antiporter subunit A	2.99	2.70	3.12
MSMEG_5328	MSMEG_5328	hypothetical protein	2.98	2.96	2.64
MSMEG_4936	atpD	ATP synthase F1, beta subunit, EC Number - 3.6.3.14	2.98	3.08	2.99
MSMEG_5422	MSMEG_5422	nucleoside triphosphate pyrophosphohydrolase	2.98	2.32	2.58
MSMEG_1529	MSMEG_1529	serine esterase, cutinase family protein	2.98	2.82	3.06
MSMEG_1008	MSMEG_1008	hypothetical protein, similar to the proteins: MSMEG_2288	2.97	2.49	2.92
MSMEG_5836	MSMEG_5836	hypothetical protein	2.97	2.57	3.01
MSMEG_0945	MSMEG_0945	hypothetical protein	2.97	3.74	2.68

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_2839	MSMEG_2839	transcriptional accessory protein	2.97	2.83	3.10
MSMEG_2364	gatC	glutamyl-tRNAGln amidotransferase, C subunit, EC Number - 6.3.5.-	2.97	2.50	2.57
MSMEG_5156	MSMEG_5156	base excision DNA repair protein, HhH-GPD family protein	2.97	2.94	2.97
MSMEG_3325	MSMEG_3325	hypothetical protein	2.96	3.13	2.65
MSMEG_4629	folC	hypothetical protein	2.96	2.79	3.07
MSMEG_2931	thrS	threonyl-tRNA synthetase, EC Number - 6.1.1.3	2.96	2.77	2.67
MSMEG_3690	MSMEG_3690	sodium:solute symporter	2.96	2.73	3.13
MSMEG_1808	MSMEG_1808	Fe-S metabolism associated SufE	2.96	2.85	2.98
MSMEG_4560	MSMEG_4560	periplasmic binding protein	2.96	2.52	2.67
MSMEG_3491	MSMEG_3491	hypothetical protein	2.95	2.87	2.68
MSMEG_4958	lysA	diaminopimelate decarboxylase, EC Number - 4.1.1.20	2.95	3.34	2.95
MSMEG_1889	MSMEG_1889	putative ribosome small subunit-dependent GTPase RsgA	2.95	2.73	2.88
MSMEG_3147	moxR	ATPase, MoxR family protein	2.94	2.69	2.92
MSMEG_2746	MSMEG_2746	conserved hypothetical alanine and leucine rich protein	2.94	3.04	3.00
MSMEG_1915	MSMEG_1915	anti-sigma factor, family protein	2.94	2.99	2.79
MSMEG_6042	MSMEG_6042	transcriptional regulator, TetR family protein	2.93	3.20	2.95
MSMEG_1245	MSMEG_1245	phosphoadenosine phosphosulfate reductase	2.93	2.91	2.40
MSMEG_2575	MSMEG_2575	hypothetical protein	2.93	2.42	2.60
MSMEG_0528	MSMEG_0528	hypothetical protein	2.92	2.27	2.19
MSMEG_0030	pknA	serine,threonine protein kinase PknA, EC Number - 2.7.1.-	2.92	2.97	3.22
MSMEG_6405	MSMEG_6405	Erp protein	2.92	2.97	3.46
MSMEG_5126	MSMEG_5126	FO synthase, EC Number - 2.5.1.-	2.90	2.83	2.80
MSMEG_1999	MSMEG_1999	hypothetical protein	2.90	2.55	1.87
MSMEG_3306	MSMEG_3306	zinc-binding alcohol dehydrogenase	2.90	2.79	2.83
MSMEG_2690	MSMEG_2690	DNA translocase FtsK	2.89	2.68	2.80
MSMEG_3068	MSMEG_3068	hypothetical protein	2.89	2.44	2.50
MSMEG_0791	thiO	glycine oxidase ThiO, EC Number - 1.4.3.19	2.88	3.00	3.04
MSMEG_3082	MSMEG_3082	soul heme-binding protein	2.88	2.93	2.72
MSMEG_0844	MSMEG_0844	putative monovalent cation,H+ antiporter subunit F	2.87	2.59	3.27
MSMEG_6286	MSMEG_6286	aspartate transaminase	2.87	2.71	2.63
MSMEG_5240	MSMEG_5240	fumarate hydratase class II, EC Number - 4.2.1.2	2.87	2.65	2.82
MSMEG_4164	MSMEG_4164	3-hydroxybutyryl-CoA dehydratase	2.87	2.73	2.49
MSMEG_3897	MSMEG_3897	proteasome component	2.87	2.79	2.86
MSMEG_3790	MSMEG_3790	RNA methyltransferase, TrmH family protein	2.86	2.64	2.72
MSMEG_5104	MSMEG_5104	tetrahydropicolinate succinylase	2.86	3.13	2.76
MSMEG_5771	MSMEG_5771	hypothetical protein	2.85	2.48	2.51
MSMEG_5800	purF	amidophosphoribosyltransferase, EC Number - 2.4.2.14	2.85	2.31	2.55
MSMEG_0940	MSMEG_0940	hypothetical protein	2.85	2.67	2.96
MSMEG_4470	MSMEG_4470	ABC transporter	2.84	2.57	2.62
MSMEG_0034	MSMEG_0034	FHA domain protein	2.84	2.76	3.18

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_3226	MSMEG_3226	glutamate synthase, NADH,nadph, small subunit	2.84	9.55	2.90
MSMEG_2010	MSMEG_2010	stress responsive A,B Barrel Domain superfamily protein	2.84	3.12	2.24
MSMEG_1996	MSMEG_1996	N5,N10- methylenetetrahydromethanopterin reductase-related protein	2.84	2.73	2.57
MSMEG_4497	MSMEG_4497	PhoH family protein	2.83	2.62	2.60
MSMEG_1679	MSMEG_1679	AmiB	2.83	2.93	2.43
MSMEG_0006	gyrA	DNA gyrase, A subunit, EC Number - 5.99.1.3	2.83	2.95	2.87
MSMEG_5447	MSMEG_5447	dolichyl-phosphate-mannose-protein mannosyltransferase	2.83	3.12	2.85
MSMEG_4298	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase, EC Number - 2.1.2.11	2.82	2.88	2.07
MSMEG_2976	hisS	histidyl-tRNA synthetase, EC Number - 6.1.1.21	2.82	2.56	2.71
MSMEG_4178	MSMEG_4178	flavodoxin,nitric oxide synthase	2.82	2.44	2.40
MSMEG_5720	MSMEG_5720	putative 3-hydroxyacyl-CoA dehydrogenase	2.82	3.04	3.14
MSMEG_5583	MSMEG_5583	HNH endonuclease	2.81	2.59	2.76
MSMEG_4626	MSMEG_4626	ribonuclease, Rne,Rng family, EC Number - 3.1.4.-	2.81	2.55	2.72
MSMEG_1809	MSMEG_1809	putative thiosulfate sulfurtransferase, EC Number - 2.8.1.1	2.81	2.68	2.54
MSMEG_4585	rbsK	ribokinase, EC Number - 2.7.1.15	2.81	2.25	2.43
MSMEG_6557	MSMEG_6557	dehydrogenase	2.81	2.80	3.06
MSMEG_2740	lexA	LexA repressor, EC Number - 3.4.21.88	2.81	2.11	1.68
MSMEG_4939	MSMEG_4939	ATP synthase delta chain, EC Number - 3.6.3.14	2.81	2.58	2.86
MSMEG_2766	MSMEG_2766	hypothetical protein	2.80	2.64	2.44
MSMEG_3647	MSMEG_3647	forkhead-associated protein	2.79	2.57	2.68
MSMEG_0638	MSMEG_0638	conserved hypothetical proline and threonine rich protein	2.79	2.06	2.64
MSMEG_6249	MSMEG_6249	hypothetical protein	2.79	2.46	2.77
MSMEG_2380	MSMEG_2380	sugar transporter family protein	2.79	2.60	2.91
MSMEG_6392	MSMEG_6392	polyketide synthase	2.78	3.06	2.70
MSMEG_2655	MSMEG_2655	LppU protein	2.78	2.78	2.99
MSMEG_5515	purH	bifunctional purine biosynthesis protein PurH, EC Number - 2.1.2.3 3.5.4.10	2.78	2.66	2.48
MSMEG_3538	MSMEG_3538	cyclopropane-fatty-acyl-phospholipid synthase 1, EC Number - 2.1.1.79	2.78	2.94	2.19
MSMEG_0946	MSMEG_0946	NAD dependent epimerase,dehydratase family protein	2.78	3.16	3.00
MSMEG_1734	MSMEG_1734	hypothetical protein	2.78	2.83	2.46
MSMEG_2457	MSMEG_2457	hypothetical protein	2.77	2.45	2.35
MSMEG_6404	glf	UDP-galactopyranose mutase, EC Number - 5.4.99.9	2.77	2.98	2.72
MSMEG_5702	MSMEG_5702	molybdenum cofactor synthesis domain protein	2.77	17.22	2.83
MSMEG_3745	MSMEG_3745	MutT,nudix family protein	2.77	2.36	2.52
MSMEG_3773	argD	acetylornithine aminotransferase, EC Number - 2.6.1.11	2.77	2.48	2.70
MSMEG_4584	proA	gamma-glutamyl phosphate reductase, EC Number - 1.2.1.41	2.76	2.65	2.81
MSMEG_5791	MSMEG_5791	hypothetical protein	2.76	2.59	2.82
MSMEG_0970	MSMEG_0970	phosphoglycerate mutase family protein	2.76	3.12	3.17
MSMEG_0035	MSMEG_0035	FHA domain protein	2.75	2.62	2.91

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_5224	ispH	4-hydroxy-3-methylbut-2-enyl diphosphate reductase, EC Number - 1.17.1.2	2.75	4.11	2.51
MSMEG_6329	MSMEG_6329	hypothetical protein	2.74	2.62	2.77
MSMEG_5362	MSMEG_5362	hypothetical protein	2.74	2.64	2.71
MSMEG_1926	MSMEG_1926	phosphoglycerate mutase family protein	2.74	2.55	2.35
MSMEG_5252	coaA	pantothenate kinase, EC Number - 2.7.1.33	2.74	2.35	2.35
MSMEG_3042	MSMEG_3042	PyrR bifunctional protein, EC Number - 2.4.2.9	2.73	2.38	2.25
MSMEG_4294	glnA	glutamine synthetase, type I, EC Number - 6.3.1.2	2.72	2.80	2.78
MSMEG_2426	MSMEG_2426	nitrogen regulatory protein P-II	2.72	2.63	2.26
MSMEG_6524	MSMEG_6524	ABC Polyamine,Opine,Phosphonate transporter, periplasmic ligand binding protein	2.72	2.97	3.08
MSMEG_4561	MSMEG_4561	ABC Fe3+-siderophores transporter, periplasmic binding protein	2.72	2.56	2.59
MSMEG_2359	MSMEG_2359	methionine synthase, vitamin-B12 independent	2.71	2.40	2.40
MSMEG_4479	MSMEG_4479	hypothetical protein	2.71	2.87	2.60
MSMEG_5119	pruA	1-pyrroline-5-carboxylate dehydrogenase, EC Number - 1.5.1.12	2.70	3.12	2.88
MSMEG_0713	MSMEG_0713	transcriptional regulator, MerR family protein	2.70	20.39	2.99
MSMEG_3645	MSMEG_3645	hypothetical protein	2.70	2.95	2.38
MSMEG_2934	MSMEG_2934	lipid A biosynthesis lauroyl acyltransferase	2.70	2.23	2.76
MSMEG_3043	pyrB	aspartate carbamoyltransferase, EC Number - 2.1.3.2	2.70	2.61	2.52
MSMEG_0642	MSMEG_0642	hypothetical ABC transporter permease protein YliD	2.70	2.67	2.61
MSMEG_2842	ssuD	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.-	2.70	2.36	2.13
MSMEG_4737	MSMEG_4737	hypothetical protein	2.70	3.13	2.88
MSMEG_3493	MSMEG_3493	putative secreted protein	2.69	2.49	2.57
MSMEG_0556	MSMEG_0556	ABC transporter, nucleotide binding,ATPase protein	2.69	2.74	3.01
MSMEG_1942	MSMEG_1942	integral membrane protein	2.69	2.39	2.53
MSMEG_1941	MSMEG_1941	helicase, UvrD,Rep family protein	2.68	2.34	2.59
MSMEG_5660	MSMEG_5660	ABC transporter ATP-binding protein	2.68	2.46	2.69
MSMEG_4215	MSMEG_4215	phosphoribosyltransferase	2.68	2.45	2.87
MSMEG_6064	MSMEG_6064	lipoprotein	2.68	4.13	2.63
MSMEG_4535	MSMEG_4535	glycoside hydrolase	2.68	5.84	2.60
MSMEG_2407	MSMEG_2407	morphine 6-dehydrogenase, EC Number - 1.1.1.218	2.68	2.16	2.52
MSMEG_3742	scpA	segregation and condensation protein A	2.67	2.57	2.29
MSMEG_6456	MSMEG_6456	hypothetical protein	2.67	3.57	2.18
MSMEG_5830	MSMEG_5830	29 kda antigen Cfp29	2.67	2.72	2.13
MSMEG_2389	hup	DNA-binding protein HU	2.66	2.56	2.51
MSMEG_5792	MSMEG_5792	hypothetical protein	2.66	2.90	2.32
MSMEG_0639	MSMEG_0639	oligopeptide transport ATP-binding protein AppF	2.66	2.77	2.46
MSMEG_0736	MSMEG_0736	hypothetical protein	2.66	2.56	2.46
MSMEG_1269	MSMEG_1269	Ser,Thr protein phosphatase family protein	2.66	2.63	2.61
MSMEG_3894	MSMEG_3894	proteasome alpha subunit	2.65	2.82	2.63
MSMEG_4908	MSMEG_4908	endo-type 6-aminohexanoate oligomer hydrolase	2.65	3.37	2.71
MSMEG_2396	MSMEG_2396	hypothetical protein	2.64	2.31	2.55

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_5807	MSMEG_5807	D-methionine transport ATP-binding protein MetN	2.64	2.78	2.43
MSMEG_0504	MSMEG_0504	carbohydrate kinase	2.64	2.27	2.82
MSMEG_3528	MSMEG_3528	ErfK,YbiS,YcfS,YnhG family protein	2.64	2.74	2.47
MSMEG_1824	MSMEG_1824	transcriptional regulator, LytR family protein	2.63	2.48	2.42
MSMEG_4736	MSMEG_4736	hypothetical protein	2.63	2.81	2.78
MSMEG_2658	MSMEG_2658	beta-lactamase	2.63	2.32	2.41
MSMEG_4389	MSMEG_4389	monooxygenase, NtaA,SnaA,SoxA family protein	2.62	2.41	2.32
MSMEG_1513	MSMEG_1513	hypothetical protein	2.62	2.70	2.99
MSMEG_0537	MSMEG_0537	hypothetical protein	2.62	9.17	3.46
MSMEG_3906	MSMEG_3906	tRNA adenine-N1--methyltransferase, EC Number - 2.1.1.36	2.62	5.62	2.64
MSMEG_5114	MSMEG_5114	acyl-CoA synthetase	2.62	2.40	2.51
MSMEG_0646	MSMEG_0646	putative transporter	2.61	3.30	2.60
MSMEG_5428	arsC	arsenate reductase, EC Number - 1.20.4.1	2.61	2.66	2.60
MSMEG_3618	MSMEG_3618	alanine and proline-rich secreted protein apa	2.61	2.50	2.39
MSMEG_0935	MSMEG_0935	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase, EC Number - 5.4.2.1	2.61	2.91	2.73
MSMEG_5774	MSMEG_5774	tRNA-dihydrouridine synthase, putative, EC Number - 1.-.-.-	2.61	2.56	2.81
MSMEG_0550	MSMEG_0550	sulfonate binding protein	2.61	2.86	3.03
MSMEG_0243	MSMEG_0243	hypothetical protein	2.60	2.27	2.40
MSMEG_4956	thrC	threonine synthase, EC Number - 4.2.3.1	2.60	2.53	2.59
MSMEG_6288	MSMEG_6288	hypothetical protein	2.60	2.65	2.62
MSMEG_2420	MSMEG_2420	hypothetical protein	2.60	2.70	2.52
MSMEG_4321	MSMEG_4321	hypothetical protein	2.60	2.64	2.38
MSMEG_6944	MSMEG_6944	hypothetical protein	2.60	2.41	2.53
MSMEG_6761	MSMEG_6761	glycerol-3-phosphate dehydrogenase 2, EC Number - 1.1.99.5	2.59	2.89	2.75
MSMEG_3643	MSMEG_3643	hypothetical protein	2.59	1.74	2.12
MSMEG_6670	MSMEG_6670	ABC transporter, permease protein, putative	2.58	3.27	2.83
MSMEG_6189	MSMEG_6189	transcriptional regulator, Crp,Fnr family protein	2.58	2.36	2.63
MSMEG_1725	MSMEG_1725	putative transposase, similar to the proteins: MSMEG_1863	2.58	2.77	3.10
MSMEG_5785	MSMEG_5785	probable exported protein	2.58	2.31	2.63
MSMEG_3121	MSMEG_3121	DNA-binding protein	2.57	2.21	2.23
MSMEG_4189	cysS	cysteinyl-tRNA synthetase, EC Number - 6.1.1.16	2.57	2.50	2.61
MSMEG_2800	MSMEG_2800	NADPH-dependent fmn reductase	2.56	2.63	2.33
MSMEG_4248	MSMEG_4248	1-acylglycerol-3-phosphate O-acyltransferase	2.56	2.30	2.16
MSMEG_4232	MSMEG_4232	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase	2.56	2.16	2.67
MSMEG_3152	hemH	ferrochelatase, EC Number - 4.99.1.1	2.56	2.13	2.29
MSMEG_2451	MSMEG_2451	HAD-superfamily protein hydrolase, subfamily protein IIA	2.56	2.30	2.08
MSMEG_1959	MSMEG_1959	hypothetical protein	2.56	2.24	2.22
MSMEG_1807	MSMEG_1807	acetyl-,propionyl-coenzyme A carboxylase alpha chain, EC Number - 6.3.4.14	2.56	2.41	2.65
MSMEG_2355	MSMEG_2355	hypothetical protein	2.55	2.47	2.43
MSMEG_2540	pyrH	uridylate kinase, EC Number - 2.7.4.-	2.54	7.50	2.63

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_2379	leuB	3-isopropylmalate dehydrogenase, EC Number - 1.1.1.85	2.54	2.26	2.40
MSMEG_3380	MSMEG_3380	pyridoxamine 5'-phosphate oxidase-related, FMN-binding	2.54	2.57	2.24
MSMEG_0694	MSMEG_0694	hypothetical protein	2.54	2.92	2.27
MSMEG_1669	sdhB	succinate dehydrogenase, iron-sulfur protein, EC Number - 1.3.99.1	2.54	2.72	2.46
MSMEG_3746	pyrG	CTP synthase, EC Number - 6.3.4.2	2.54	2.42	2.34
MSMEG_3622	MSMEG_3622	hypothetical protein	2.53	2.23	2.04
MSMEG_4485	glyS	glycyl-tRNA synthetase, EC Number - 6.1.1.14	2.53	2.36	2.38
MSMEG_2905	MSMEG_2905	transcriptional regulator	2.53	2.54	2.44
MSMEG_0024	MSMEG_0024	peptidyl-prolyl cis-trans isomerase B, EC Number - 5.2.1.8	2.53	2.75	2.80
MSMEG_4498	MSMEG_4498	hypothetical protein	2.53	2.65	2.69
MSMEG_3305	MSMEG_3305	integral membrane protein DUF6	2.53	2.76	2.15
MSMEG_4583	MSMEG_4583	ATPase associated with various cellular activities	2.52	2.09	2.42
MSMEG_3674	MSMEG_3674	hypothetical protein	2.52	2.59	2.15
MSMEG_4323	MSMEG_4323	pyruvate dehydrogenase E1 component, EC Number - 1.2.4.1	2.52	2.55	2.57
MSMEG_2961	secD	preprotein translocase subunit SecD	2.52	2.37	2.56
MSMEG_2448	MSMEG_2448	hypothetical protein	2.51	2.25	2.15
MSMEG_4572	MSMEG_4572	hypothetical protein	2.51	2.45	2.75
MSMEG_1178	MSMEG_1178	transcriptional regulator	2.51	2.07	2.18
MSMEG_4269	asnB	asparagine synthase glutamine-hydrolyzing, EC Number - 6.3.5.4	2.51	2.24	2.23
MSMEG_3671	MSMEG_3671	integral membrane protein	2.51	2.41	2.46
MSMEG_5257	MSMEG_5257	channel protein, hemolysin III family protein	2.51	2.31	2.28
MSMEG_3036	nusB	transcription antitermination protein NusB	2.51	1.87	2.05
MSMEG_3233	MSMEG_3233	cytochrome D ubiquinol oxidase subunit 1, EC Number - 1.10.3.-	2.51	2.26	2.31
MSMEG_4694	MSMEG_4694	HNH endonuclease family protein	2.50	4.01	2.67
MSMEG_2693	MSMEG_2693	competence-damaged protein	2.50	2.21	2.57
MSMEG_3100	opcA	OpcA protein	2.50	2.70	2.64
MSMEG_1670	sdhA	succinate dehydrogenase, flavoprotein subunit, EC Number - 1.3.99.1	2.50	2.78	2.65
MSMEG_4688	MSMEG_4688	hypothetical protein	2.50	2.33	2.20
MSMEG_1395	MSMEG_1395	lipoprotein, putative	2.49	2.38	2.52
MSMEG_1998	MSMEG_1998	hydrolase, alpha,beta fold family protein	2.49	2.53	2.23
MSMEG_1244	MSMEG_1244	hypothetical protein	2.49	3.75	2.35
MSMEG_1316	prfC	peptide chain release factor 3	2.49	2.54	2.70
MSMEG_3774	argB	acetylglutamate kinase, EC Number - 2.7.2.8	2.49	2.42	2.65
MSMEG_2528	MSMEG_2528	glycerate kinase	2.49	2.05	2.44
MSMEG_0690	MSMEG_0690	iron-sulfur cluster-binding protein	2.49	2.09	2.62
MSMEG_0250	MSMEG_0250	membrane protein, MmpL family protein	2.49	2.30	2.86
MSMEG_3641	MSMEG_3641	hypothetical protein	2.49	2.30	2.68
MSMEG_0843	MSMEG_0843	putative monovalent cation,H+ antiporter subunit G	2.49	2.13	2.57
MSMEG_3776	argC	N-acetyl-gamma-glutamyl-phosphate reductase, EC Number - 1.2.1.38	2.48	2.31	2.36

Probe Name	Gene Name	Description	Fold Change		
			Wayne	DPI	AA
MSMEG_1583	groL	chaperonin GroEL	2.48	2.37	2.53
MSMEG_6894	rplI	50S ribosomal protein L9	2.48	2.28	2.79
MSMEG_4256	MSMEG_4256	NLP,P60 family protein	2.48	2.23	2.24
MSMEG_2731	MSMEG_2731	ATPase involved in DNA repair	2.48	3.23	2.71
MSMEG_3385	MSMEG_3385	hypothetical protein	2.48	2.26	1.94
MSMEG_5086	MSMEG_5086	very-long-chain acyl-CoA synthetase, EC Number - 6.2.1.- 6.2.1.3	2.47	2.31	2.20
MSMEG_2922	MSMEG_2922	hypothetical protein	2.47	2.36	2.11
MSMEG_3142	MSMEG_3142	HTH-type transcriptional repressor AcnR	2.47	2.44	2.21
MSMEG_1832	MSMEG_1832	hypothetical protein	2.47	2.21	2.31
MSMEG_1015	MSMEG_1015	transcriptional regulator, TetR family protein, similar to the proteins: MSMEG_2295	2.47	2.58	2.88
MSMEG_4899	rdgB	non-canonical purine NTP pyrophosphatase, RdgB,HAM1 family protein	2.47	2.03	2.26
MSMEG_3447	MSMEG_3447	two-component system response regulator	2.47	17.85	2.70
MSMEG_3330	MSMEG_3330	hypothetical protein	2.47	2.58	2.51
MSMEG_4972	MSMEG_4972	acetyltransferase	2.47	2.50	2.55
MSMEG_4399	MSMEG_4399	hypothetical protein	2.46	2.18	2.52
MSMEG_3184	treZ	malto-oligosyltrehalose trehalohydrolase, EC Number - 3.2.1.141	2.45	2.26	2.34
MSMEG_3033	aroB	3-dehydroquinate synthase, EC Number - 4.2.3.4	2.44	2.13	2.31
MSMEG_5070	MSMEG_5070	Trypsin	2.44	2.23	2.17
MSMEG_4531	cysW	sulfate ABC transporter, permease protein CysW	2.44	2.13	2.26
MSMEG_3887	tatA	twin arginine-targeting protein translocase, TatA,E family protein	2.43	2.28	2.07
MSMEG_4192	MSMEG_4192	hypothetical protein	2.43	2.35	2.17
MSMEG_1599	MSMEG_1599	RNA polymerase sigma factor SigD	2.43	2.71	2.34
MSMEG_4391	MSMEG_4391	acyl-CoA dehydrogenase-family protein	2.43	2.19	2.02
MSMEG_4180	hisG	ATP phosphoribosyltransferase, EC Number - 2.4.2.17	2.43	2.44	2.53
MSMEG_3117	MSMEG_3117	cytochrome aa3 controlling protein	2.43	2.36	2.56
MSMEG_4190	MSMEG_4190	inositol monophosphatase family protein	2.43	2.36	2.41
MSMEG_0928	murB	UDP-N-acetylenolpyruvoylglucosamine reductase, EC Number - 1.1.1.158	2.43	2.30	2.25
MSMEG_0402	MSMEG_0402	linear gramicidin synthetase subunit D, EC Number - 5.1.1.- 1.-.-.-	2.43	2.61	2.47
MSMEG_0918	MSMEG_0918	transcriptional regulator, XRE family protein	2.42	2.75	2.60
MSMEG_2581	MSMEG_2581	acetyltransferase	2.42	2.29	2.30
MSMEG_5968	MSMEG_5968	polysaccharide biosynthesis protein	2.41	2.80	2.43
MSMEG_1653	MSMEG_1653	hypothetical protein	2.41	2.50	2.36
MSMEG_6391	MSMEG_6391	propionyl-CoA carboxylase beta chain, EC Number - 6.4.1.3	2.40	2.58	2.61
MSMEG_1242	iscS	cysteine desulfurase IscS, EC Number - 4.4.1.-	2.40	5.41	2.32
MSMEG_1528	MSMEG_1528	cutinase, EC Number - 3.1.1.74	2.40	2.34	2.40
MSMEG_4571	rpsT	30S ribosomal protein S20	2.40	2.40	2.33
MSMEG_5029	MSMEG_5029	alkanal monooxygenase alpha chain	2.40	2.39	2.70

4.3.1.4 List of pathways expressed under all three different conditions

98 pathways were identified using kegg pathway database which were differentially expressed under all three conditions.

Number of genes showing altered expression ratios, categorized according to the pathway they belong to, are displayed in Table 4.4. The results clearly show that the pathways related to protein synthesis are mostly less functional. Majority of genes belonging to Amino-acyl tRNA biosynthesis, protein export, purine and pyrimidine metabolism, Ribososme, RNA polymerase, RNA degradation pathways are down-regulated.

Table 4.4: List of pathways differentially expressed under all three conditions, obtained from kegg pathways(Expression ratio above 1.5 fold)

GO_id	PATHWAYS	No. Of genes down-regulated in all 3 conditions	No. Of genes up-regulated in all 3 conditions
msm02010	ABC transporters	30	15
msm00250	Alanine, aspartate and glutamate metabolism	16	6
msm00520	Amino sugar and nucleotide sugar metabolism	2	1
msm00970	Aminoacyl-tRNA biosynthesis	19	1
msm00627	Aminobenzoate degradation	18	14
msm00330	Arginine and proline metabolism	16	13
msm03410	Base excision repair	2	1
msm00410	beta-Alanine metabolism	8	17
msm01110	Biosynthesis of secondary metabolites	123	66
msm01040	Biosynthesis of unsaturated fatty acids	41	4
msm00020	Citrate cycle (TCA cycle)	15	2
msm03030	DNA replication	5	1
msm00061	Fatty acid biosynthesis	5	5
msm00071	Fatty acid metabolism	8	22
msm00260	Glycine, serine and threonine metabolism	18	3
msm00010	Glycolysis / Gluconeogenesis	13	18
msm00630	Glyoxylate and dicarboxylate metabolism	11	11
msm00340	Histidine metabolism	17	8
msm00562	Inositol phosphate metabolism	4	2
msm00300	Lysine biosynthesis	11	4
msm00310	Lysine degradation	9	13
msm03430	Mismatch repair	5	1
msm00910	Nitrogen metabolism	10	8
msm03420	Nucleotide excision repair	7	1
msm00190	Oxidative phosphorylation	20	5

msm00770	Pantothenate and CoA biosynthesis	8	4
msm00030	Pentose phosphate pathway	11	2
msm00360	Phenylalanine metabolism	3	3
msm00400	Phenylalanine, tyrosine and tryptophan	11	4
msm00640	Propanoate metabolism	14	17
msm00230	Purine metabolism	37	10
msm00240	Pyrimidine metabolism	25	3
msm00620	Pyruvate metabolism	10	10
msm03010	Ribosome	53	4
msm03018	RNA degradation	8	2
msm00521	Streptomycin biosynthesis	2	3
msm00380	Tryptophan metabolism	11	12
msm02020	Two-component system	15	6
msm00350	Tyrosine metabolism	3	12
msm00290	Valine, leucine and isoleucine biosynthesis	13	2
msm00280	Valine, leucine and isoleucine degradation	14	15
msm00592	alpha-Linolenic acid metabolism	0	1
msm00053	Ascorbate and aldarate metabolism	0	8
msm00550	Peptidoglycan biosynthesis	6	0
msm03060	Protein export	7	0
msm03020	RNA polymerase	4	0
msm00920	Sulfur metabolism	6	0
msm04122	Sulfur relay system	7	0
msm05152	Tuberculosis	6	0
msm00750	Vitamin B6 metabolism	4	0
msm00480	Glutathione metabolism	6	0
msm03440	Homologous recombination	8	0
msm00401	Novobiocin biosynthesis	1	0
msm03070	Bacterial secretion system	12	0
msm00270	Cysteine and methionine metabolism	16	0

4.3.1.5 Pathways and the genes involved in those pathways along with the expression ratios in three different conditions.

Table 4.5 Details of selected pathways

A: Glycolysis/Gluconeogenesis		Down-regulated		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
MSMEG_1926	phosphoglycerate mutase family protein	2.74	2.35	2.55
lpdA	dihydrolipoamide dehydrogenase, EC Number - 1.8.1.4	1.71	1.77	1.95
MSMEG_0935	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase, EC Number - 5.4.2.1	2.61	2.73	2.91
MSMEG_1762	piperideine-6-carboxylic acid dehydrogenase	1.73	1.79	1.75
tpiA	triosephosphate isomerase, EC Number - 5.3.1.1	6.24	4.90	7.10
Pyk	pyruvate kinase, EC Number - 2.7.1.40	3.55	3.27	3.61
Pgk	phosphoglycerate kinase, EC Number - 2.7.2.3	15.29	11.04	18.21
MSMEG_4579	phosphoglycerate mutase family protein	1.92	1.78	1.75

MSMEG_4323	pyruvate dehydrogenase E1 component, EC Number - 1.2.4.1	2.52	2.57	2.55
MSMEG_6088	phosphoglycerate mutase	1.84	2.07	1.59
glpX	fructose-1,6-bisphosphatase, class II, EC Number - 3.1.3.11	3.90	3.65	3.83
Eno	enolase, EC Number - 4.2.1.11	2.31	2.31	2.02
		Up-regulated		
		Wayne	**DPI**	**AA**
MSMEG_6242	alcohol dehydrogenase, iron-containing, EC Number - 1.1.1.1	1.83	1.72	1.63
MSMEG_1158	5-oxovalerate dehydrogenase	1.64	1.44	1.56
MSMEG_1543	eptc-inducible aldehyde dehydrogenase, EC Number - 1.2.1.3	1.80	1.76	1.90
MSMEG_0900	eptc-inducible aldehyde dehydrogenase, EC Number - 1.2.1.3	1.88	1.71	1.89
MSMEG_5650	AMP-dependent synthetase and ligase	1.63	1.49	1.60
MSMEG_6834	alcohol dehydrogenase	1.90	1.68	1.96
MSMEG_3388	S-hydroxymethylglutathione dehydrogenase,class III alcohol dehydrogenase, EC Number - 1.1.1.284 1.1.1.1	1.57	1.66	1.33
MSMEG_0671	S-hydroxymethylglutathione dehydrogenase, EC Number -	1.71	1.52	1.50
MSMEG_6833	alcohol dehydrogenase, EC Number - 1.1.1.1	2.24	2.07	2.18
MSMEG_0217	alcohol dehydrogenase B, EC Number - 1.1.1.1	1.64	1.59	1.40
MSMEG_6338	phosphoglycerate mutase family protein, putative	1.64	1.46	1.67
MSMEG_3986	acetyl-coenzyme A synthetase, EC Number - 6.2.1.1	1.73	1.80	1.73
MSMEG_0127	oxidoreductase, zinc-binding dehydrogenase family protein	1.53	1.36	1.53
MSMEG_5859	aldehyde dehydrogenase NAD family protein, EC Number -	1.99	1.78	1.92
MSMEG_6297	aldehyde dehydrogenase	1.65	1.45	1.65
MSMEG_1461	5-oxovalerate dehydrogenase	1.63	1.56	1.59
MSMEG_5866	alcohol dehydrogenase B, EC Number - 1.1.1.1	2.06	2.06	1.36
MSMEG_1138	alcohol dehydrogenase 1, EC Number - 1.1.1.1	1.61	1.64	1.79

Table 4.5 B. Glyoxylate and dicarboxylate metabolism		**Down-regulated**		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
aceA	isocitrate lyase, EC Number - 4.1.3.1	1.83	2.07	5.19
Eda	2-dehydro-3-deoxyphosphogluconate aldolase,4-hydroxy-2-oxoglutarate aldolase, EC Number - 4.1.2.14 4.1.3.16	2.21	2.48	2.17
purU	formyltetrahydrofolate deformylase, EC Number - 3.5.1.10	1.89	1.78	1.86
MSMEG_2528	glycerate kinase	2.49	2.44	2.05
MSMEG_1813	propionyl-CoA carboxylase beta chain, EC Number - 6.4.1.3	4.68	3.65	4.68
gltA	citrate synthase I, EC Number - 4.1.3.7	4.60	4.68	4.56
MSMEG_4668	oxidoreductase alpha molybdopterin subunit	4.36	4.20	3.70
MSMEG_5273	beta-ketoadipyl CoA thiolase, EC Number - 2.3.1.-	2.24	2.14	2.27
mce	methylmalonyl-CoA epimerase, EC Number - 5.1.99.1	3.23	2.94	3.21
acnA	aconitate hydratase 1, EC Number - 4.2.1.3	5.00	4.07	11.95
glcB	malate synthase G, EC Number - 2.3.3.9	5.82	5.12	5.69
		Up-regulated		
		Wayne	**DPI**	**AA**
MSMEG_5478	hydroxypyruvate isomerase, putative	1.67	1.53	1.67
gcl	glyoxylate carboligase, EC Number - 4.1.1.47	1.81	1.78	1.83
MSMEG_5477	2-hydroxy-3-oxopropionate reductase, EC Number - 1.1.1.60	1.83	1.49	2.01
MSMEG_4035	citrate synthase	1.78	1.90	1.80

MSMEG_5297	oxalyl-CoA decarboxylase, EC Number - 4.1.1.8	1.58	1.54	1.76
MSMEG_0157	oxalyl-CoA decarboxylase, EC Number - 4.1.1.8	2.10	1.86	1.83
MSMEG_0161	formate dehydrogenase, alpha subunit, EC Number - 1.2.1.2	1.90	1.76	1.85
MSMEG_0159	MSMEG_0159	2.14	2.01	1.22
MSMEG_2254	oxalate decarboxylase OxdC, putative, EC Number - 4.1.1.2	1.82	2.00	2.12
MSMEG_1986	tartrate dehydrogenase, EC Number - 1.1.1.93	1.71	1.71	1.64

Table 4.5 C. TCA Cycle		**Down-regulated**		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
MSMEG_1671	**succinate dehydrogenase hydrophobic membrane anchor protein SdhD**	**4.40**	**4.17**	**6.90**
sdhA	succinate dehydrogenase, flavoprotein subunit, EC Number - 1.3.99.1	2.50	2.65	2.78
lpdA	dihydrolipoamide dehydrogenase, EC Number - 1.8.1.4	1.71	1.77	1.95
sdhB	succinate dehydrogenase, iron-sulfur protein, EC Number - 1.3.99.1	2.54	2.46	2.72
MSMEG_1654	isocitrate dehydrogenase, NADP-dependent, EC Number - 1.1.1.42	4.19	3.89	4.01
pyc	pyruvate carboxylase, EC Number - 6.4.1.1	3.54	3.51	3.07
acnA	**aconitate hydratase 1, EC Number - 4.2.1.3**	**5.00**	**4.07**	**11.95**
MSMEG_4323	pyruvate dehydrogenase E1 component, EC Number - 1.2.4.1	2.52	2.57	2.55
sucB	**2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase, EC Number - 2.3.1.61**	**3.65**	**3.50**	**13.63**
sucC	succinyl-CoA synthetase, beta subunit, EC Number - 6.2.1.5	15.72	12.23	16.87
MSMEG_5240	fumarate hydratase class II, EC Number - 4.2.1.2	2.87	2.82	2.65
sucD	**succinyl-CoA synthetase, alpha subunit, EC Number - 6.2.1.5**	**13.31**	**11.53**	**60.61**
gltA	citrate synthase I, EC Number - 4.1.3.7	4.60	4.68	4.56
orB	2-oxoglutarate ferredoxin oxidoreductase subunit beta	6.34	5.36	6.01
MSMEG_4646	pyruvate synthase	3.98	3.93	3.94
		Up-regulated		
		Wayne	**DPI**	**AA**
MSMEG_4035	[2Fe-2S] binding domain protein	1.78	1.90	1.80
MSMEG_0418	shikimate kinase, EC Number - 2.7.1.71	1.74	1.53	1.68

Table 4.5 D. Oxidative phosphorylation		**Up-regulated**		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
MSMEG_1671	**succinate dehydrogenase hydrophobic membrane anchor protein SdhD**	**4.40**	**4.17**	**6.90**

sdhA	succinate dehydrogenase, flavoprotein subunit, EC Number - 1.3.99.1	2.50	2.65	2.78
sdhB	succinate dehydrogenase, iron-sulfur protein, EC Number - 1.3.99.1	2.54	2.46	2.72
MSMEG_3117	cytochrome aa3 controlling protein	2.43	2.56	2.36
ndh	**NADH dehydrogenase, EC Number - 1.6.99.3**	**9.20**	**8.42**	**19.14**
MSMEG_4268	cytoChrome c oxidase subunit 2, EC Number - 1.9.3.1	8.37	6.20	8.76
MSMEG_4260	cytoChrome c oxidase subunit 3, EC Number - 1.9.3.1	15.95	8.87	15.72
MSMEG_4263	ubiquinol-cytochrome c reductase cytochrome b subunit	3.84	3.60	3.84
MSMEG_4261	**ubiquinol-cytochrome c reductase cytochrome c subunit**	**8.63**	**7.93**	**561.27**
MSMEG_4262	ubiquinol-cytochrome c reductase iron-sulfur subunit	11.23	8.90	11.00
cydB	ATP synthase F0, C subunit, EC Number - 3.6.3.14	3.07	2.46	3.15
AtpE	ATP synthase F1, alpha subunit, EC Number - 3.6.3.14	1.80	1.58	1.61
atpA	ATP synthase F0, C subunit, EC Number - 3.6.3.14	2.16	2.12	2.11
atpB	ATP synthase F1, epsilon subunit, EC Number - 3.6.3.14	9.15	7.29	9.91
atpC	ATP synthase F1, gamma subunit, EC Number - 3.6.3.14	5.52	5.40	5.87
atpG	ATP synthase F1, beta subunit, EC Number - 3.6.3.14	10.75	10.13	10.34
atpD	ATP synthase subunit beta	2.98	2.99	3.08
		Up-regulated		
		Wayne	**DPI**	**AA**
MSMEG_0418	succinate dehydrogenase flavoprotein subunit, EC Number - 1.3.99.1	1.74	1.53	1.68
MSMEG_4433	dehydrogenase	1.74	1.76	2.13
nuoM	NADH dehydrogeanse subunit M	1.57	1.52	1.53
nuoI	RNA polymerase sigma-70 factor	2.08	1.90	1.25
nuoF	NADH-quinone oxidoreductase, F subunit, EC Number - 1.6.99.5	1.62	1.69	1.53

Table 4.5 E. Fatty Acid Biosynthesis		**Down-regulated**		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
MSMEG_1807	acetyl-,propionyl-coenzyme A carboxylase alpha chain, EC Number - 6.3.4.14	2.56	2.65	2.41
MSMEG_4325	malonyl CoA-acyl carrier protein transacylase, EC Number - 2.3.1.39	4.86	3.96	4.84
MSMEG_5248	acyl-[ACP] desaturase	5.89	5.32	5.63
MSMEG_5773	fatty acid desaturase	3.49	3.28	3.45
		Up-regulated		
		Wayne	**DPI**	**AA**
MSMEG_4835	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	1.78	1.67	1.68
MSMEG_6011	short chain dehydrogenase	1.72	1.75	1.82
MSMEG_0269	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number -	1.67	1.50	1.75

Table 4.5 E. Fatty Acid Biosynthesis		Down-regulated		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
	1.1.1.100			
MSMEG_1490	3-oxoacyl-ACP synthase III	1.62	1.55	1.23
MSMEG_4169	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	1.77	1.92	2.04

Table 4.5 F. fatty Acid Metabolism		Down-regulated		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
MSMEG_4254	AMP-binding enzyme	2.25	2.14	2.07
MSMEG_3139	enoyl-CoA hydratase	3.39	3.23	3.78
MSMEG_4164	3-hydroxybutyryl-CoA dehydratase	2.87	2.49	2.73
MSMEG_6353	delta3,5-delta2,4-dienoyl-CoA isomerase, EC Number -	1.82	1.88	1.86
MSMEG_5720	putative 3-hydroxyacyl-CoA dehydrogenase	2.82	3.14	3.04
MSMEG_5273	beta-ketoadipyl CoA thiolase, EC Number - 2.3.1.-	2.24	2.14	2.27
MSMEG_5277	probable enoyl-CoA hydratase, EC Number - 4.2.1.17	2.00	2.04	1.82
MSMEG_1048	probable enoyl-CoA hydratase, EC Number - 4.2.1.17, similar to the proteins: MSMEG_2328	1.82	1.67	1.63
MSMEG_1762	piperideine-6-carboxylic acid dehydrogenase	1.73	1.79	1.75
MSMEG_2080	putative acyl-CoA dehydrogenase	3.39	3.35	3.22
		Up-regulated		
		Wayne	**DPI**	**AA**
MSMEG_6242	alcohol dehydrogenase, iron-containing, EC Number - 1.1.1.1	1.83	1.72	1.63
MSMEG_1158	5-oxovalerate dehydrogenase	1.64	1.44	1.56
MSMEG_1543	eptc-inducible aldehyde dehydrogenase, EC Number - 1.2.1.3	1.80	1.76	1.90
MSMEG_0900	eptc-inducible aldehyde dehydrogenase, EC Number - 1.2.1.3	1.88	1.71	1.89
MSMEG_6834	alcohol dehydrogenase	1.90	1.68	1.96
MSMEG_3388	dihydropyrimidinase, EC Number - 3.5.2.2	1.57	1.66	1.33
MSMEG_0671	S-hydroxymethyl glutathione dehydrogenase, EC Number -	1.71	1.52	1.50
MSMEG_6833	alcohol dehydrogenase, EC Number - 1.1.1.1	2.24	2.07	2.18
MSMEG_0217	alcohol dehydrogenase B, EC Number - 1.1.1.1	1.64	1.59	1.40
MSMEG_6774	enoyl-CoA hydratase	1.64	1.47	1.48
MSMEG_3012	acetyl-CoA acetyltransferases	1.66	1.44	1.55
MSMEG_0259	enoyl-CoA hydratase,isomerase	2.03	1.70	2.03
MSMEG_2229	enoyl-CoA hydratase	1.81	1.61	2.04
MSMEG_0127	oxidoreductase, zinc-binding dehydrogenase family protein	1.53	1.36	1.53
MSMEG_5859	aldehyde dehydrogenase NAD family protein, EC Number -	1.99	1.78	1.92
MSMEG_6297	aldehyde dehydrogenase	1.65	1.45	1.65
MSMEG_1461	5-oxovalerate dehydrogenase	1.63	1.56	1.59
MSMEG_5866	alcohol dehydrogenase B, EC Number - 1.1.1.1	2.06	2.06	1.36
MSMEG_1138	alcohol dehydrogenase 1, EC Number - 1.1.1.1	1.61	1.64	1.79
MSMEG_2948	6-oxocamphor hydrolase	1.57	1.50	1.57
MSMEG_5915	enoyl-CoA hydratase	2.06	1.80	2.07

Table 4.5 G. Nitrogen Metabolism		Down-regulated		
Gene Name	**Description**	**Wayne**	**DPI**	**AA**
MSMEG_2394	putative cystathionine gamma-synthase, EC Number - 2.5.1.48	2.04	2.01	1.75
MSMEG_4527	sulfite reductase [ferredoxin], EC Number - 1.8.7.1	6.28	6.28	6.51
asnB	asparagine synthase glutamine-hydrolyzing, EC Number - 6.3.5.4	2.51	2.23	2.24
gcvT	glycine cleavage system T protein, EC Number - 2.1.2.10	1.77	1.90	1.79
MSMEG_3225	ferredoxin-dependent glutamate synthase 1, EC Number - 1.4.7.1	4.35	4.15	4.30
MSMEG_3226	**glutamate synthase, NADH,nadph, small subunit**	**2.84**	**2.90**	**9.55**
glnA	glutamine synthetase, type I, EC Number - 6.3.1.2	2.72	2.78	2.80
MSMEG_6082	carbonic anhydrase	4.38	4.34	4.97
MSMEG_6458	glutamate synthase subunit beta	1.64	1.82	1.59
		Up-regulated		
		Wayne	**DPI**	**AA**
MSMEG_5374	glutamate--ammonia ligase	1.85	1.89	1.64
MSMEG_5373	nitrilase 2, EC Number - 3.5.5.1	2.15	2.01	1.06
MSMEG_0332	2-nitropropane dioxygenase, NPD	1.87	1.86	2.05
narI	respiratory nitrate reductase, gamma subunit, EC Number - 1.7.99.4	1.71	1.87	2.03
MSMEG_5140	nitrate reductase, alpha subunit, EC Number - 1.7.99.4	1.96	2.01	2.09
nirB	nitrite reductase [NADPH], large subunit, EC Number - 1.7.1.4	1.75	1.65	1.74
glnT	glutamine synthetase, type III, EC Number - 6.3.1.2	1.92	1.73	1.92

4.3.1.6 Description of pathways commonly up and down regulated under all three conditions

Metabolic Pathways & ATP Synthesis

In persistent phase the metabolism is changed from glucose to lipids, glycolysis is decreased and the glyoxylate shunt is upregulated allowing anaplerotic maintenance of the tricarboxylic acid (TCA) cycle [21, 22 & 24]. We obtained 3 fold down regulation in the expression of enzyme pyruvate kinase under all three conditions as well as down regulation of enzymes phosphoglycerate kinase and phosphoglycerate mutase. This shows that glycolysis is down regulated under these three conditions. Many dehydrogenases like alcohol dehydrogenases, aldehyde dehydrogenases and oxovalerate dehydrogenases are up-regulated. This might be needed to maintain the NADH pool under dormant conditions. 15 of 17 genes in TCA cycle are down regulated indicating general slowdown of metabolism. This involves genes like succinate dehydrogenase, isocitrate dehydrogenase, pyruvate carboxylase,

aconitate hydratase, pyruvate dehydrogenase etc. Surprisingly, the icl gene which encodes isocitrate lyase is also down regulated in all three models. The glcB gene which encodes malate synthase is down regulated. 20 of 25 genes belonging to oxidative phosphyration are down regulated. Genes such as NADH dehydrogenase, succinate dehydrogenase, ubiquinol cytochrome C reductase, cytochrome C oxidase are all down regulated under all three conditions indicating shut down of Electron transport chain.

It should be noted that some genes from metabolic pathways are down regulated at a greater extent in ascorbic acid (AA) induced dormancy model compared to Wayne's dormancy model and DPI induced dormancy model (Highlighted in Table 4.5). This includes genes from TCA cycle like succinate dehydrogenase, aconitate hydratase, 2-oxoglutarate dehydrogenase and succinyl CoA synthetase. Also, genes encoding NADH dehydrogenase and ubiquinol-cytochrome c reductase cytochrome c subunit are highly down regulated in AA induced dormancy model. As cytochrome c reductase is down regulated by 561 fold in AA induced dormancy model shows less requirement of this enzyme. It is involved in ATP generation.

ATP synthase, an essential enzyme in the energy metabolism, is a validated drug target for the treatment of tuberculosis, and ATP synthase inhibitors, such as diarylquinoline, are promising candidate drugs for the treatment of infections caused by *M. tuberculosis* [25]. ATP synthase is a multi-subunit complex consisting of a membrane-embedded F_0 part and a cytosolic F_1 moiety. The enzyme can utilize the proton-motive force (PMF) across the bacterial cytoplasmatic membrane for the synthesis of ATP. At low PMF, for example in environments with limited oxygen concentrations, this reaction can be reversed in several bacteria, which use the energy released from hydrolysis of ATP to maintain a PMF [26]. A strong down-regulation of ATP synthase is characteristic of dormant bacilli in different models [27]. We found down-regulation of ATP synthase subunit namely, atpA, atpB, atpC, atpD, atpE, atpG and cydB under all three conditions. This shows the importance of ATP synthesis in active growth whereas it is not required during dormancy.

In fatty acid biosynthesis, 4 genes are down regulated namely, acetyl-propionyl-coenzyme A carboxylase, malonyl CoA-acyl carrier protein transacylase and 2 desaturases whereas, 5 genes are up regulated namely, 3 3-oxacyl reductases

and 3-oxacyle ACP synthase. In fatty acid metabolism, 10 are down regulated whereas 21 are up regulated. This shows the importance of fatty acid metabolism during conversion to dormancy. Enoyl CoA hydratase is up-regulated. It hydrates double bond between second and third carbons on acyl CoA. It is essential to produce acetyl CoA and energy. Thus, it could be important in dormancy for energy production. Acetyl CoA acetyl transferase which catalyses the reaction between 2-acetyl CoA and acetyl CoA, is up-regulated. Thus, fatty acids are used as energy sources under dormancy.

Nitrogen Metabolism

Mycobacteria shift to nitrogen metabolism during dormancy is a hallmark event [28-30]. Nitrate reductase (subunit narI 1.7.99.4) and nitrite reductase (nirB 1.7.1.15) were highly up-regulated (Figure 4.6). Thus, these results show importance of nitrogen metabolism during dormancy. Gene encoding glutamate synthase is highly down regulated in AA induced dormancy model than in other two models. Therefore, it is mainly required during active growth of *M. smegmatis* bacilli.

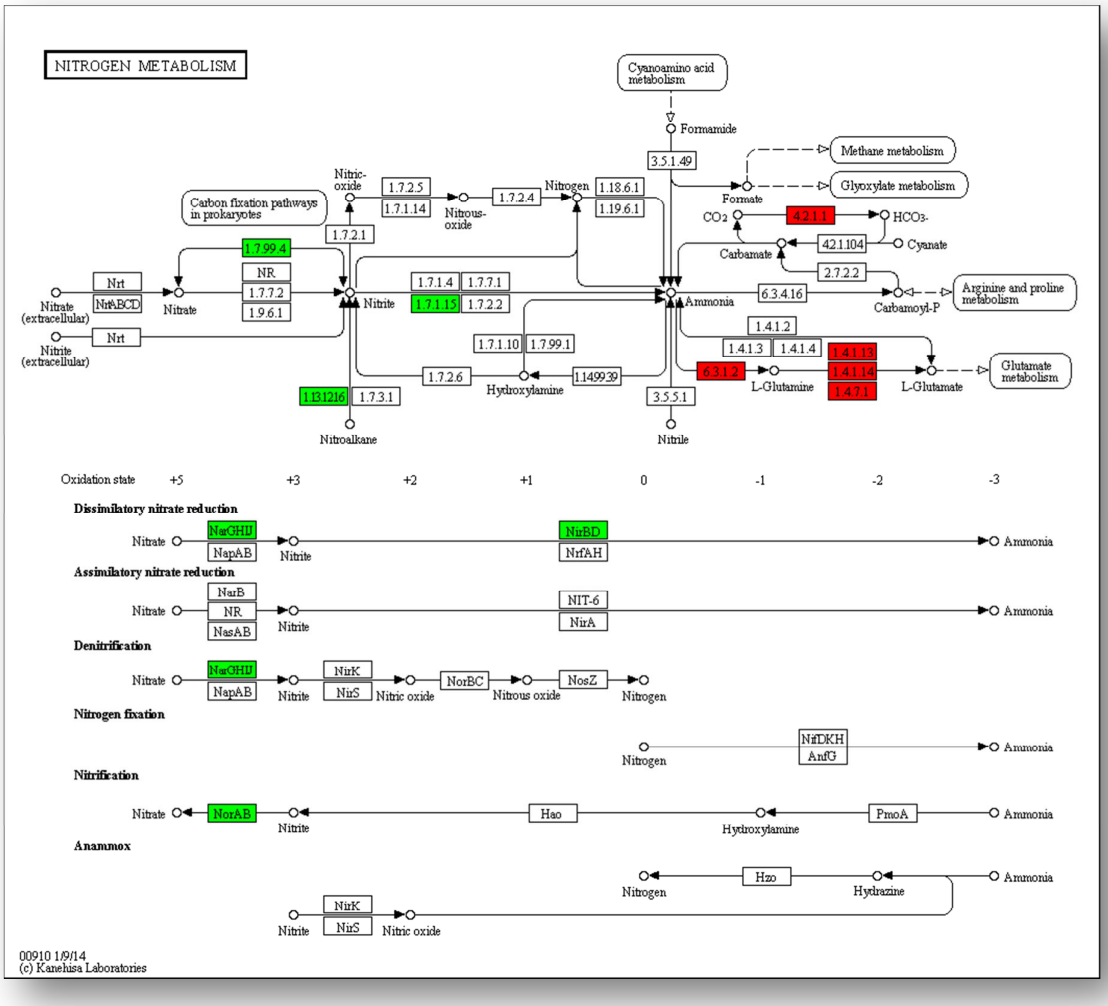


Figure 4.3: Kegg Pathway showing up and down regulated genes in Nitrogen Metabolism. Green box represents genes which are up-regulated while red box represents genes which are down regulated.

Protein, RNA and Ribosome

We found pathways related to protein synthesis are highly down-regulated. 19 of 20 genes from aminoacyl tRNA biosynthesis pathway are down-regulated which involves enzymes like Prolyl tRNA synthetase (proS), tryptophanyl tRNA synthetase (trpS), alanine tRNA synthetase (alaS), histidyl tRNA synthetase (hisS), aspartyl tRNA synthetase (aspS), phenylalanyl tRNA synthetase (pheS), glycyl tRNA synthetase (glyS), isoleucil tRNA synthetase (ileS) and threonyl tRNA synthetase (thrS). All these Aminoacyl-tRNA synthetases (aaRSs) catalyze the aminoacylation of their cognate tRNAs through a two-step mechanism involving activation of the amino

acid by ATP to yield the aminoacyl-adenylate, followed by transfer to the 3'terminal adenosine of the tRNA molecule [31], ensuring translation of the genetic code for respective amino acid [32]. All genes in protein export are down-regulated (7 of 7 genes) like preprotein translocases (secG, secA2, sec D, tatC, tatA). 37 out of 47 genes fron purine metabolism are down-regulated and 25 out of 28 genes from pyrimidine metabolism are down-regulated. GTP pyrophospokinase which catalyzes the formation of pppGpp which is then hydrolysed to form ppGpp is down-regulated. pppGpp acts as a signalling molecule to control bacterial gene expression involved in long term survival in starvation [33]. DNA directed RNA polymerase is also down-regulated. Guanine deaminase is up-regulated which is involved in guanine catabolism and thus helps to maintain the purine pool [34]. Cytidylate kinase which catalyzes the reaction between ATP and ADP and thus maintains the pool of ATP is also down-regulated. 53 out of 57 genes from Ribosome are down-regulated whereas all genes from RNA polymerase are down-regulated. Overall, protein synthesis, protein export, ribosome assembly, purine and pyrimidine metabolism are down-regulated under dormancy.

Amino acids

16 out of 22 genes from alanine, aspartate and glutamate metabolism are down-regulated which includes enzymes like arginosuccinate lyase (argH) and arginosuccinate synthase (argG). ArgG produces argininosuccinate which is then converted into arginine by argH. L-aspartate oxidase that catalyzes the reaction between oxaloacetate and aspartate is also down-regulated. Other enzymes such as glutamine synthetase, aspargine synthase, glutamate synthase are also down-regulated under all three conditions. All genes from cysteine and methionine metabolism are down-regulated (16 out of 16). 17 out of 25 genes from lysine biosynthesis are down-regulated whereas 13 out of 22 genes from lysine degradation are up-regulated. 18 out of 21 genes from gycine, threonine and serine metabolism are down-regulated. Overall, it is quite apparent that most of the amino acid synthesis is down-regulated which corroborates with the results obtained for protein synthesis expression ratios.

Peptidoglycan Synthesis

All genes from peptidoglycan synthesis are down-regulated (6 of 6 genes). This pathway include genes like UDP-N-acetylmuramoylalanine-D-glutamate ligase (murD 6.3.2.9), phospho-N-acetylmuramoyl-pentapeptide-transferase (mraY 2.7.8.13), UDP-N-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase (6.3.2.13) which are required for peptidoglycan synthesis. Thus, it is clear that peptidoglycan synthesis is shut down during dormancy (Figure 4.4).

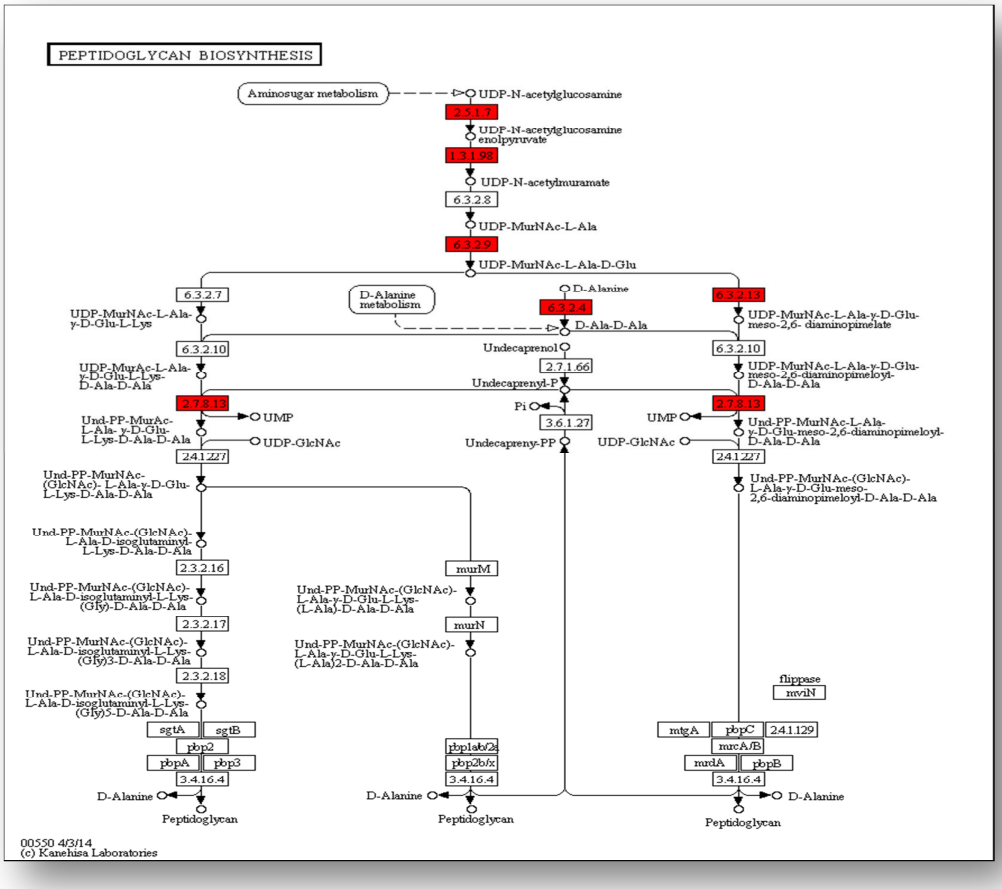


Figure 4.4: Kegg Pathway showing up and down regulated genes in Peptidoglycan synthesis pathway. Green box represents genes which are up-regulated while red box represents genes which are down regulated.

DNA replication

6 out of 7 genes from DNA replication are down-regulated which include enzyme like DNA polymerase III alpha subunit and DnaG (Figure 4.5)

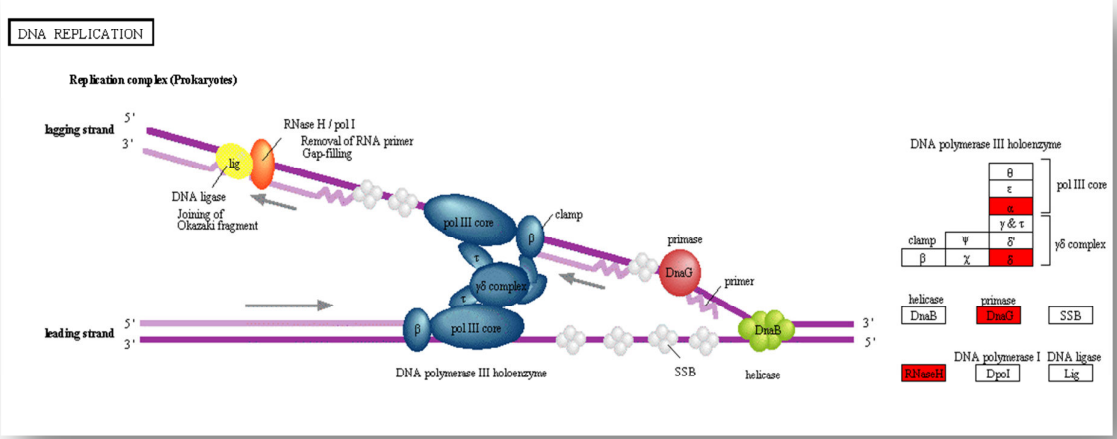


Figure 4.5: Kegg Pathway showing up and down regulated genes in DNA replication pathway. Green box represents genes which are up-regulated while red box represents genes which are down regulated.

ABC Transporters

30 of 45 genes from ABC transporters family are down-regulated. Genes encoding sulfate transporters (cysA, cysW and cysT) are down-regulated. ABC transporter ATP binding protein, ABC transporter permease protein, ABC transporter membrane spanning protein, phosphate ABC transporter (pst A, pstB, pstC and pstS) are all down-regulated under all three conditions. This shows that the transport system of mycobacteria down-regulated under dormancy. On the other hand, genes such as ABC type drug export system membrane protein, ABC type drug export system ATP binding protein, bacterial extracellular solute binding protein, sugar ABC transporters are all up-regulated. The differential expression of ABC transporters reflect the presence of selective mechanisms mycobacteria possess under dormant conditions.

Ascorbate and Alderate Metabolism

All the genes from ascorbate and alderate metabolism are up-regulated (8 out of 8). This includes genes like 5-oxovalerate dehydrogenase, eptc-inducible aldehyde dehydrogenase, glucarate dehydratase, aldehyde dehydrogenase NAD family protein, aldehyde dehydrogenase and UDP-glucose 6-dehydrogenase. It is earlier shown that

many aldehyde dehydrogenases are up-regulated during dormancy in Mtb, but its role is still not clear [35].

4.3.1.7 Important genes exclusively up-regulated in DPI treated sample

Table 4.6: List of genes exclusively up-regulated in DPI treated sample (Expression ratio >1.5)

Gene Name	Description	fold change
MSMEG_2495	decarboxylase	5.31
MSMEG_2211	DNA-binding protein	4.58
MSMEG_6176	2-dehydro-3-deoxy-6-phosphogalactonate aldolase, EC Number - 4.1.2.21	4.38
MSMEG_2475	putative oxidoreductase YdbC, EC Number - 1.-.-.-	4.28
MSMEG_3830	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	3.98
MSMEG_1433	thioesterase	3.91
MSMEG_4292	integral membrane protein	3.70
MSMEG_1192	M23 peptidase domain protein	3.43
mmsA	methylmalonate-semialdehyde dehydrogenase, EC Number - 1.2.1.27	2.81
MSMEG_3997	regulatory protein	2.63
MSMEG_2720	NADH ubiquinone oxidoreductase, 20 kda subunit	2.61
MSMEG_6069	CobW,P47K C- domain protein	2.51
MSMEG_6645	2-methylcitrate dehydratase 2, EC Number - 4.2.1.79	2.49
MSMEG_4764	ABC transporter permease protein	2.42
MSMEG_2891	biphenyl-2,3-diol 1,2-dioxygenase 1, EC Number - 1.13.11.39	2.39
MSMEG_0144	HNH endonuclease	2.37
MSMEG_5106	HNH endonuclease	2.33
MSMEG_5052	DNA-binding protein	2.30
MSMEG_2100	peptidase family M20,M25,M40, EC Number - 3.4.-.-	2.25
MSMEG_5632	oxidoreductase, short chain dehydrogenase,reductase family protein	2.21
MSMEG_5761	cupin domain protein	2.17
MSMEG_6870	creatinine amidohydrolase	2.14
MSMEG_4616	GAF family protein	2.14
MSMEG_5996	acetyl-CoA acetyltransferase	2.12
MSMEG_2061	NADH-quinone oxidoreductase chain c, EC Number - 1.6.99.5	2.05
MSMEG_0838	AsnC-family protein transcriptional regulator	2.05
MSMEG_4213	cytochrome P450 hydroxylase	2.03
MSMEG_6911	ABC transporter, ATP-binding protein GluA	1.92
MSMEG_5621	putative acyl-CoA dehydrogenase	1.88
MSMEG_2461	transporter permease 2, putative	1.88
MSMEG_4076	butyryl-CoA dehydrogenase	1.86
MSMEG_0335	enoyl-CoA hydratase,isomerase	1.85
MSMEG_2194	MerR-family protein transcriptional regulator	1.85
MSMEG_1291	putative oxidoreductase	1.84
pcaC	4-carboxymuconolactone decarboxylase domain protein, EC Number - 4.1.1.44	1.84
MSMEG_5724	bacterial regulatory protein, MarR family protein	1.83
MSMEG_2354	oxidoreductase	1.83

Gene Name	Description	fold change
MSMEG_6856	MmgE,PrpD family protein	1.83
MSMEG_1617	kumamolisin	1.79
MSMEG_0237	ISMsm3, transposase	1.78
MSMEG_0568	radical SAM domain protein	1.77
MSMEG_2202	dimethylaniline monooxygenase [N-oxide-forming] 5, EC Number - 1.14.13.8	1.76
MSMEG_0502	glucosidase	1.76
MSMEG_5124	2,4-dienoyl-coA reductase	1.74
MSMEG_4952	acyl-CoA synthetase	1.74
MSMEG_6795	enoyl-CoA hydratase,isomerase family protein	1.74
MSMEG_1462	ferredoxin	1.74
MSMEG_2825	IS1549, transposase	1.73
MSMEG_3818	thermolabile glutaminase, EC Number - 3.5.1.2	1.73
hslR	heat shock protein 15	1.73
MSMEG_5644	membrane protein	1.73
MSMEG_6855	carboxyvinyl-carboxyphosphonate phosphorylmutase, EC Number - 2.7.8.23	1.72
MSMEG_4862	3-alpha-hydroxysteroid dehydrogenase	1.72
MSMEG_3318	oxidoreductase	1.71
ilvD	dihydroxy-acid dehydratase, EC Number - 4.2.1.9	1.71
MSMEG_6002	coenzyme A transferase, subunit A	1.71
MSMEG_6662	short chain dehydrogenase	1.71
MSMEG_6322	bifunctional wax ester synthase, acyl-CoA diacylglycerol acyltransferase	1.71
amt	ammonium transporter	1.71
MSMEG_2507	IclR-family protein transcriptional regulator	1.70
MSMEG_3763	ABC transporter efflux protein, DrrB family protein	1.70
MSMEG_0126	mandelate racemase,muconate lactonizing enzyme	1.70
speB	agmatinase, EC Number - 3.5.3.11	1.70
MSMEG_6151	alpha,beta hydrolase fold-1	1.70
MSMEG_3957	conserved 13e12 repeat family protein	1.70
MSMEG_5596	oxidoreductase	1.70
MSMEG_5190	TetR-family protein transcriptional regulator	1.69
MSMEG_0573	putative ECF sigma factor RpoE1	1.69
MSMEG_2085	NADPH-ferredoxin reductase fpra, EC Number - 1.18.1.2	1.69
MSMEG_4136	dTDP-glucose 4,6-dehydratase	1.69
MSMEG_0622	putative DNA-binding protein	1.69
MSMEG_4882	short chain dehydrogenase	1.68
MSMEG_3975	regulatory protein, putative	1.68
MSMEG_3571	thioesterase family protein	1.67
MSMEG_0606	transcriptional regulator, TetR family protein	1.67
MSMEG_2034	aryl-alcohol dehydrogenase, EC Number - 1.1.1.90	1.67
MSMEG_5358	acetamidase,Formamidase family protein	1.66
MSMEG_1636	RNA methyltransferase, TrmH family protein, group 2	1.66
MSMEG_5163	ABC proline,glycine betaine family protein transporter, periplasmic ligand	1.66
MSMEG_4140	GntR-family protein transcriptional regulator	1.66
MSMEG_1901	DNA-binding HTH domain containing protein, putative	1.66
MSMEG_0277	putative aminotransferase, EC Number - 2.6.1.-	1.66
MSMEG_2911	integral membrane transport protein	1.66
MSMEG_2535	dehydrogenase,reductase SDR family member 10, EC Number - 1.1.-.-	1.65

Gene Name	Description	fold change
MSMEG_4983	transcriptional regulator, TetR family protein	1.65
MSMEG_2233	acyl-CoA dehydrogenase, C- domain protein	1.65
eutB	ethanolamine ammonia-lyase, large subunit, EC Number - 4.3.1.7	1.65
MSMEG_5165	integral membrane protein	1.65
MSMEG_4077	enoyl-CoA hydratase	1.65
MSMEG_4339	tetratricopeptide repeat family protein	1.65
MSMEG_0697	integral membrane protein	1.64
MSMEG_6532	transcriptional regulatory protein	1.64
MSMEG_4039	aryl-alcohol dehydrogenase, EC Number - 1.1.1.90	1.64
MSMEG_0746	carbon-monoxide dehydrogenase, large subunit, EC Number - 1.2.99.2	1.64
MSMEG_0131	acyl-CoA synthetase	1.64
MSMEG_4812	respiratory-chain NADH dehydrogenase domain, 51 kda subunit	1.63
MSMEG_6562	putative acyl-CoA dehydrogenase	1.63
MSMEG_0715	3-oxoacyl-[acyl-carrier-protein] reductase, EC Number - 1.1.1.100	1.63
MSMEG_1895	HTH-type transcriptional regulator AlsR	1.63
MSMEG_2147	gp15 protein	1.63
MSMEG_4117	2-deoxy-D-gluconate 3-dehydrogenase, EC Number - 1.1.1.125	1.63
pcaC	4-carboxymuconolactone decarboxylase, EC Number - 4.1.1.44	1.63
MSMEG_2342	putative glycosyltransferase	1.63
MSMEG_5881	putative carbon monoxide dehydrogenase subunit G	1.63
MSMEG_4511	linear gramicidin synthetase subunit B, EC Number - 5.1.1.- 5.1.1.-	1.63
MSMEG_0048	pyridoxamine 5'-phosphate oxidase family protein	1.63
MSMEG_4516	2,3-dihydroxybenzoate-AMP ligase, EC Number - 6.3.2.-	1.63
MSMEG_3372	transcriptional regulator, ArsR family protein	1.63
MSMEG_5338	regulatory protein, MarR	1.62
MSMEG_1690	putative ECF sigma factor RpoE1	1.62
MSMEG_0747	carbon monoxide dehydrogenase F protein	1.62
MSMEG_5948	glycosyl transferase	1.62
MSMEG_1851	Sec tRNA	1.62
MSMEG_1968	putative oxidoreductase, EC Number - 1.-.-.-	1.62
MSMEG_1213	cytochrome P450 monooxygenase	1.62
MSMEG_1464	translation initiation inhibitor	1.62
MSMEG_5719	oxidoreductase	1.62
MSMEG_6852	putative carboxylesterase,lipase	1.61
MSMEG_6736	LacI-family protein transcriptional regulator	1.61
MSMEG_6295	shikimate transporter	1.61
MSMEG_6169	type II,IV secretion system protein	1.61
MSMEG_5913	dioxygenase	1.61
MSMEG_4713	HpcH,HpaI aldolase,citrate lyase family protein	1.61
MSMEG_5795	4-amino-4-deoxychorismate lyase	1.61
MSMEG_5590	carboxylate-amine ligase Nfa27300, EC Number - 6.3.-.-	1.61
dapB	dihydrodipicolinate reductase, N-terminus domain protein, EC Number -	1.61
MSMEG_2499	ABC transporter, membrane spanning protein, putative	1.61
MSMEG_1274	gluconolactonase	1.60
MSMEG_0062	ftsk,spoiiie family protein	1.60
MSMEG_0503	DeoR-family protein transcriptional regulator	1.60
MSMEG_1329	major facilitator superfamily protein MFS_1, putative	1.60

Gene Name	Description	fold change
MSMEG_0718	acetyl-CoA synthetase	1.60
MSMEG_2214	alcohol dehydrogenase B, EC Number - 1.1.1.1	1.60
MSMEG_0663	TetR-family protein transcriptional regulator	1.60
MSMEG_5911	AraC family protein transcriptional regulator	1.59
MSMEG_3516	3-alpha-or 20-beta-hydroxysteroid dehydrogenase	1.59
MSMEG_6341	6-phosphogluconate dehydrogenase, NAD-binding	1.59
MSMEG_0896	integral membrane transport protein	1.59
MSMEG_2170	3-hydroxyisobutyrate dehydrogenase family protein	1.59
MSMEG_3989	peptidase M20D, amidohydrolase	1.59
MSMEG_5495	enoyl-CoA hydratase	1.59
MSMEG_0020	Periplasmic binding protein	1.59
cbiQ	cobalt ABC transporter, permease protein CbiQ	1.59
MSMEG_1097	glycosyl transferase, group 2 family protein	1.59
MSMEG_1893	UbiE,COQ5 methyltransferase	1.59
MSMEG_4442	zinc-binding oxidoreductase	1.59
MSMEG_5337	amidate substrates transporter protein	1.59
MSMEG_6775	major facilitator superfamily protein transporter	1.59
MSMEG_2810	major facilitator superfamily protein	1.59
mdcH	malonate decarboxylase, epsilon subunit	1.58
MSMEG_0362	amidohydrolase 2	1.58
MSMEG_6764	transcriptional regulator, TetR family protein	1.58
MSMEG_3256	mucin-associated surface protein	1.58
MSMEG_6872	beta-lactamase	1.58
MSMEG_6326	Ser tRNA	1.58
MSMEG_5186	HicB family protein	1.58
eda	2-dehydro-3-deoxyphosphogluconate aldolase,4-hydroxy-2-oxoglutarate aldolase, EC Number - 4.1.2.14 4.1.3.16	1.58
MSMEG_5912	succinic semialdehyde dehydrogenase	1.58
MSMEG_6471	glycine,D-amino acid oxidase	1.58
MSMEG_0721	integral membrane protein	1.58
MSMEG_6503	transcriptional regulator, TetR family protein	1.58
MSMEG_6642	caib,baif family protein	1.58
MSMEG_0042	TetR-family protein transcriptional regulator	1.58
MSMEG_6818	dehydrogenase	1.57
MSMEG_1482	methyltransferase	1.57
MSMEG_3288	LysM domain protein	1.57
MSMEG_5869	putative short chain dehydrogenase	1.57
MSMEG_1795	2-deoxy-scyllo-inosamine dehydrogenase, EC Number - 1.1.1.-	1.57
nuoG	NADH-quinone oxidoreductase, G subunit, EC Number - 1.6.99.5	1.57
MSMEG_1153	FAD dependent oxidoreductase	1.57
MSMEG_1777	UsfY protein	1.57
MSMEG_5640	amylo-alpha-1,6-glucosidase	1.57
MSMEG_2567	probable monooxygenase, EC Number - 1.14.13.-	1.57
MSMEG_0886	serine,threonine-protein kinase PknD, EC Number - 2.7.11.1	1.57
MSMEG_5577	fructokinase, EC Number - 2.7.1.4	1.56
MSMEG_3445	5,10-methylenetetrahydromethanopterin reductase, EC Number - 1.5.99.11	1.56
MSMEG_0235	probable conserved membrane protein	1.56

Gene Name	Description	fold change
MSMEG_3362	enoyl-CoA hydratase	1.56
MSMEG_4877	CaiB,BaiF family protein	1.56
MSMEG_2455	carbon monoxide dehydrogenase subunit G CoxG superfamily protein	1.56
MSMEG_6644	malyl-CoA lyase	1.56
nuoH	NADH-quinone oxidoreductase, H subunit, EC Number - 1.6.99.5	1.56
MSMEG_5024	T,U mismatch-specific DNA glycosylase	1.56
MSMEG_4656	sugar ABC transporter ATP-binding protein	1.56
MSMEG_5854	transcriptional regulator, TetR family protein	1.55
MSMEG_1222	ISMsm6, transposase	1.55
MSMEG_5380	Leu tRNA	1.55
MSMEG_1491	histidinol-phosphate aminotransferase 2, EC Number - 2.6.1.9	1.55
ureC	urease, alpha subunit, EC Number - 3.5.1.5	1.55
MSMEG_0744	carbon monoxide dehydrogenase medium chain, EC Number - 1.2.99.2	1.55
MSMEG_3373	major facilitator superfamily protein MFS_1	1.55
MSMEG_2652	iron repressor protein	1.55
rfbA	glucose-1-phosphate thymidylyltransferase, EC Number - 2.7.7.24	1.55
MSMEG_0265	uracil DNA glycosylase superfamily protein	1.55
MSMEG_4452	Met tRNA	1.55
MSMEG_0603	putative acyl-CoA dehydrogenase	1.55
MSMEG_0812	amino acid transporter	1.55
MSMEG_3554	N5,N10-methylene- tetrahydromethanopterin reductase	1.55
MSMEG_6050	solute-binding lipoprotein	1.55
MSMEG_1370	2-deoxy-scyllo-inosamine dehydrogenase, EC Number - 1.1.1.-	1.55
MSMEG_4858	transcriptional regulator, TetR family protein	1.55
MSMEG_4574	DNA-binding protein	1.55
MSMEG_4381	amidase	1.55
MSMEG_2872	short-chain dehydrogenase,reductase SDR	1.55
MSMEG_1453	Citrate transporter	1.55
MSMEG_1214	oxidoreductase	1.55
MSMEG_1710	ribose transport system permease protein RbsC, EC Number - 3.6.3.17	1.55
MSMEG_1648	putative transcriptional regulator	1.54
MSMEG_4002	oxidoreductase, zinc-binding dehydrogenase family protein	1.54
MSMEG_0306	arylamine N-acetyltransferase, EC Number - 2.3.1.5	1.54
MSMEG_1693	succinate dehydrogenase [ubiquinone] flavoprotein subunit, EC Number - 1.3.5.1	1.54
MSMEG_2216	AMP-binding enzyme	1.54
MSMEG_6052	ABC transport protein, ATP-binding subunit	1.54
MSMEG_0170	transmembrane transport protein	1.54
MSMEG_5641	glycosyl transferase, group 1 family protein	1.54
MSMEG_2024	hydroxymethylglutaryl-CoA lyase	1.54
xth	exodeoxyribonuclease III, EC Number - 3.1.11.2	1.54
MSMEG_3615	zinc-binding alcohol dehydrogenase family protein, EC Number - 1.-.-.-	1.54
MSMEG_2830	ISMsm4, transposase, similar to the proteins: MSMEG_0430 ; MSMEG_1239	1.54
MSMEG_3677	serine,threonine protein kinase	1.54
MSMEG_1796	membrane protein	1.54
MSMEG_5383	dehydrogenase,reductase SDR family member 4, EC Number - 1.1.1.184	1.54
MSMEG_1899	succinyl-CoA:3-ketoacid-coenzyme A transferase subunit B, EC Number -	1.54
MSMEG_6175	2-keto-3-deoxy-galactonokinase	1.54

Gene Name	Description	fold change
MSMEG_0574	putative ECF sigma factor RpoE1	1.54
MSMEG_3273	glutamyl aminopeptidase, M42 family protein	1.54
MSMEG_0529	probable serine,threonine-protein kinase PknK, EC Number - 2.7.11.1	1.53
MSMEG_0474	glucosamine--fructose-6-phosphate aminotransferase [isomerizing], EC Number	1.53
MSMEG_2610	cobalt transport protein ATP-binding subunit	1.53
MSMEG_5404	propionate--CoA ligase	1.53
MSMEG_5764	putative cyanamide hydratase	1.53
MSMEG_1606	benzoylformate decarboxylase, EC Number - 4.1.1.7	1.53
MSMEG_0506	ABC transporter, permease protein	1.53
MSMEG_4354	dipeptide-binding protein of ABC transport system	1.53
mdcG	phosphoribosyl-dephospho-CoA transferase MdcG, EC Number - 2.7.7.61	1.53
MSMEG_1141	ABC-transporter integral membrane protein	1.53
MSMEG_1993	MaoC like domain protein	1.53
dapB	dihydrodipicolinate reductase, N-terminus domain protein, EC Number -	1.53
MSMEG_4776	glyoxalase,bleomycin resistance protein,dioxygenase	1.53
MSMEG_2646	mini-circle putative transposase for, Transposase IS116,IS110,IS902 family	1.53
MSMEG_4684	ribose 5-phosphate isomerase, EC Number - 5.3.1.6	1.53
MSMEG_0540	transcriptional regulator, putative	1.53
MSMEG_3984	Transposase IS116,IS110,IS902 family protein	1.53
MSMEG_0376	transcriptional regulator, AraC family protein	1.53
MSMEG_0960	putative transmembrane protein	1.53
MSMEG_4509	MbtG protein	1.53
MSMEG_6299	glyoxylate reductase, EC Number - 1.1.1.26	1.53
MSMEG_6030	cytochrome p450	1.53
MSMEG_6028	transcriptional regulator, LysR family protein	1.53
MSMEG_6045	ABC heavy metal transporter, inner membrane subunit	1.53
MSMEG_5235	short chain dehydrogenase family protein	1.53
MSMEG_2565	4-hydroxy-2-oxovalerate aldolase, EC Number - 4.1.2.-	1.53
MSMEG_0270	aminoglycoside phosphotransferase	1.53
MSMEG_5802	oxidoreductase, short chain dehydrogenase,reductase family protein	1.53
MSMEG_6206	serine,threonine protein kinase PksC, EC Number - 2.7.11.1	1.53
MSMEG_3268	ABC transporter, permease protein	1.52
tilS	tRNAIle-lysidine synthetase, EC Number - 6.-.-.-	1.52
MSMEG_6506	nicotinamidase,pyrazinamidase	1.52
MSMEG_1185	transcriptional regulator, AsnC family protein	1.52
hisD	histidinol dehydrogenase, EC Number - 1.1.1.23	1.52
MSMEG_0179	transcriptional regulator, GntR family protein	1.52
MSMEG_6875	endoribonuclease L-PSP family protein	1.52
MSMEG_0876	short chain dehydrogenase	1.52
MSMEG_6605	transcriptional regulatory protein	1.52
MSMEG_6814	helix-turn-helix, Fis-type	1.52
MSMEG_2493	aminotransferase, class I and II family protein	1.52
MSMEG_0476	chitin synthase	1.52
MSMEG_5464	integral membrane transport protein	1.52
MSMEG_5920	FMN-dependent monooxygenase	1.52
MSMEG_0429	putative ferric uptake regulator	1.52
MSMEG_2014	molybdenum import ATP-binding protein ModC, EC Number - 3.6.3.29	1.52

Gene Name	Description	fold change
MSMEG_4036	crotonobetaine,carnitine-CoA ligase	1.52
MSMEG_4871	3-hydroxyacyl-CoA dehydrogenase type-2, EC Number - 1.1.1.35 1.1.1.178	1.52
95 Genes	Hypothetical Proteins	1.52-

4.3.1.8 Important genes exclusively down-regulated in DPI

Table 4.7: List of genes down-regulated exclusively in DPI treated sample(Expression ratio >1.5)

GeneName	Description	fold change
MSMEG_2439	LppW protein	62.37
MSMEG_4852	enoyl-CoA hydratase, putative	30.39
deoC	deoxyribose-phosphate aldolase, EC Number - 4.1.2.4	18.79
MSMEG_3018	transglutaminase domain protein	14.42
MSMEG_2164	transcriptional regulator, putative	13.75
thiD	phosphomethylpyrimidine kinase, EC Number - 2.7.4.7	12.85
MSMEG_2932	HIT family protein hydrolase	12.54
MSMEG_2504	3-isopropylmalate dehydratase small subunit, EC Number - 4.2.1.33	11.96
xseA	exodeoxyribonuclease VII, large subunit, EC Number - 3.1.11.6	11.73
MSMEG_3490	putative membrane acyltransferase	11.12
MSMEG_6457	oxidoreductase molybdopterin binding domain, putative	10.61
MSMEG_0676	putative transcriptional regulatory protein	9.25
MSMEG_2886	stress responsive A,B Barrel Domain family protein	8.07
MSMEG_3604	sorbitol utilization protein SOU2, EC Number - 1.1.-.-	8.02
nthA	nitrile hydratase, alpha subunit, EC Number - 4.2.1.84	7.69
MSMEG_2384	Gln tRNA	7.63
MSMEG_0871	putative aldehyde or xanthine dehydrogenase, molybdopterin binding subunit	7.58
MSMEG_4553	GAF domain, putative	7.31
MSMEG_0310	aldehyde dehydrogenase family protein	7.14
MSMEG_2929	thioesterase family protein	6.81
MSMEG_6881	transcriptional regulator, GntR family protein	6.58
MSMEG_6884	NADP oxidoreductase, coenzyme f420-dependent:6-phosphogluconate dehydrogenase, nad-binding, putative	6.49
MSMEG_4153	cytochrome P450	6.47
MSMEG_0571	hydrolase, carbon-nitrogen family protein	6.03
MSMEG_5833	phosphoribosylformylglycinamidine synthase subunit PurS	6.00
ppk	polyphosphate kinase, EC Number - 2.7.4.1	5.95
MSMEG_1296	uricase, EC Number - 1.7.3.3	5.84
MSMEG_1477	major facilitator superfamily protein	5.66
MSMEG_3238	3-mercaptopyruvate sulfurtransferase	5.60
MSMEG_0670	FAD dependent oxidoreductase	5.54
snm	secretion protein Snm4	5.45
argS	arginyl-tRNA synthetase, EC Number - 6.1.1.19	5.23
MSMEG_0432	bifunctional uroporphyrinogen-III synthetase,response regulator domain protein	5.22
MSMEG_1078	hydrolase	5.08
MSMEG_4685	oxidoreductase	5.01

GeneName	Description	fold change
MSMEG_1769	UsfY protein	4.77
MSMEG_1234	taurine import ATP-binding protein TauB, EC Number - 3.6.3.36	4.75
MSMEG_6802	ABC transporter ATP-binding protein	4.69
MSMEG_5291	acyl-CoA synthetase	4.64
MSMEG_0159	formate dehydrogenase, gamma subunit, EC Number - 1.2.1.2	4.57
MSMEG_5429	LpqT protein	4.52
hybA	hydrogenase-2, small subunit, EC Number - 1.12.99.6	4.50
MSMEG_0484	formamidase, EC Number - 3.5.1.49	4.39
MSMEG_2470	acyl-CoA thioesterase	4.31
MSMEG_3780	adenylate and Guanylate cyclase catalytic domain protein	4.30
MSMEG_5425	Gln tRNA	4.21
MSMEG_0358	ribonucleoside-diphosphate reductase, beta subunit, EC Number - 1.17.4.1	4.04
MSMEG_4953	putative transcriptional regulator	3.98
MSMEG_2984	putative hydrolase	3.95
MSMEG_1034	putative esterase family protein, similar to the proteins: MSMEG_2314	3.90
MSMEG_6004	oxidoreductase, 2-nitropropane dioxygenase family protein	3.88
recD	exodeoxyribonuclease V, alpha subunit, EC Number - 3.1.11.5	3.80
MSMEG_5823	conserved membrane-spanning protein	3.73
MSMEG_5416	LpqU protein	3.71
MSMEG_1803	RsbW protein	3.71
murG	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase, EC Number - 2.4.1.227	3.70
MSMEG_2103	5,10-methylenetetrahydromethanopterin reductase, EC Number - 1.5.99.11	3.68
MSMEG_6762	transcriptional regulator	3.60
MSMEG_1074	polysaccharide deacetylase	3.50
MSMEG_6479	putative transcriptional regulator	3.50
MSMEG_3815	possible drug efflux membrane protein	3.43
MSMEG_6563	dihydrokaempferol 4-reductase	3.36
MSMEG_3039	monooxygenase	3.34
MSMEG_0524	short chain dehydrogenase	3.32
MSMEG_2252	flavin-type hydroxylase	3.28
MSMEG_5731	transcriptional regulator, GntR family protein	3.26
MSMEG_4964	transcriptional regulator, TetR family protein	3.20
MSMEG_1678	transcriptional regulator, LysR family protein	3.17
pcaC	4-carboxymuconolactone decarboxylase domain protein, EC Number - 4.1.1.44	3.17
MSMEG_4904	rhomboid family protein	3.12
MSMEG_4709	enoyl-CoA hydratase	3.11
MSMEG_5302	aerobic C4-dicarboxylate transport protein	3.07
MSMEG_6218	secreted protein	3.06
MSMEG_1873	thymidylate kinase	3.05
MSMEG_2804	two-component system sensor kinase	3.05
MSMEG_3345	transcriptional regulator, GntR family protein	3.05
MSMEG_5310	SAM-dependent methyltransferase	3.02
MSMEG_0836	carboxylate-amine ligase ,, EC Number - 6.3.-.-	3.02
MSMEG_2405	MarR-family protein transcriptional regulator	2.97
MSMEG_3679	phosphohydrolase	2.96
MSMEG_5676	citrate synthase 2	2.94

GeneName	Description	fold change
MSMEG_0501	glucosamine-6-phosphate deaminase 1, EC Number - 3.5.99.6	2.93
MSMEG_4881	methylmalonyl-CoA mutase, N-terminus of large subunit	2.92
MSMEG_6576	pyridoxamine 5'-phosphate oxidase family protein	2.91
MSMEG_6922	transporter, major facilitator family protein	2.91
MSMEG_6664	methylenetetrahydrofolate reductase family protein, putative	2.86
MSMEG_0221	secreted protein	2.81
MSMEG_2006	2,3-dihydroxybiphenyl 1,2-dioxygenase	2.73
MSMEG_6060	permease	2.73
dinB	DNA polymerase IV, EC Number - 2.7.7.7	2.72
MSMEG_0244	two component response transcriptional regulatory protein prra	2.72
MSMEG_0561	putative TetR family protein receptor protein	2.68
MSMEG_2346	phytoene synthase, EC Number - 2.5.1.-	2.64
MSMEG_5039	Siderophore-interacting protein	2.62
MSMEG_2726	glutamate permease	2.57
MSMEG_4662	deoxyribose-phosphate aldolase superfamily protein	2.53
rfbA	glucose-1-phosphate thymidylyltransferase, EC Number - 2.7.7.24	2.52
MSMEG_5159	DNA-binding response regulator, putative	2.51
MSMEG_1050	SAM-dependent methyltransferase, similar to the proteins: MSMEG_2330	2.48
putP	sodium,proline symporter	2.44
MSMEG_0300	amidohydrolase family protein	2.41
MSMEG_6748	ATPase component of ABC transporters with duplicated ATPase domains	2.41
MSMEG_6027	major facilitator superfamily protein MFS_1	2.40
MSMEG_0889	succinic semialdehyde dehydrogenase	2.40
MSMEG_1009	cytochrome p450, similar to the proteins: MSMEG_2289	2.40
MSMEG_0289	alpha,beta hydrolase fold family protein	2.38
katA	catalase KatA, EC Number - 1.11.1.6	2.36
dnl1	DNA ligase I, ATP-dependent, EC Number - 6.5.1.1	2.35
MSMEG_4990	DNA-binding response regulator	2.33
MSMEG_0906	DNA-binding protein	2.32
glmS	glucosamine--fructose-6-phosphate aminotransferase, isomerizing, EC Number -	2.32
MSMEG_2745	thymidylate synthase	2.30
MSMEG_3464	alcohol dehydrogenase, EC Number - 1.1.1.1	2.24
MSMEG_3599	sugar-binding transcriptional regulator, LacI family protein	2.24
MSMEG_4715	acyl-CoA dehydrogenase, EC Number - 1.3.99.-	2.21
MSMEG_0393	Fmt protein	2.21
MSMEG_3973	N-methylhydantoinase	2.17
MSMEG_1412	amino acid permease	2.16
MSMEG_4046	L-carnitine dehydratase,bile acid-inducible protein F	2.16
MSMEG_4174	transcriptional regulator, IclR family protein	2.15
MSMEG_5860	transcriptional regulator, TetR family protein	2.14
MSMEG_5914	acyl-CoA synthetase	2.14
MSMEG_1621	pyrimidine-specific ribonucleoside hydrolase RihA, EC Number - 3.2.-.-	2.11
MSMEG_0650	GntR-family protein transcriptional regulator	2.06
trpD	anthranilate phosphoribosyltransferase, EC Number - 2.4.2.18	2.04
MSMEG_0124	transcriptional regulator, GntR family protein	2.01
dinB	DNA polymerase IV, EC Number - 2.7.7.7	2.01
MSMEG_4295	hydrolase, alpha,beta fold family protein	1.99

GeneName	Description	fold change
MSMEG_4149	acetaldehyde dehydrogenase, EC Number - 1.2.1.10	1.99
MSMEG_4155	transposase A	1.98
MSMEG_6543	B12 binding domain protein	1.98
MSMEG_5350	PPE family protein, putative	1.97
MSMEG_2212	short chain dehydrogenase	1.97
MSMEG_3980	transcriptional regulator, GntR family protein	1.97
MSMEG_5589	manganese transport protein MntH	1.95
MSMEG_6266	thiocyanate hydrolase beta subunit, EC Number - 3.5.5.8	1.93
MSMEG_0979	keratin associated protein	1.93
MSMEG_4550	transposase	1.93
MSMEG_2179	transcriptional regulatory protein	1.92
MSMEG_4477	hydrolase, alpha,beta hydrolase fold family protein	1.92
MSMEG_0017	ABC transporter, permease,ATP-binding protein	1.89
MSMEG_0022	L-ornithine 5-monooxygenase	1.87
MSMEG_6132	glucarate dehydratase, EC Number - 4.2.1.40	1.86
MSMEG_0539	transcriptional regulator, Crp,Fnr family protein	1.85
MSMEG_3348	bacterial extracellular solute-binding protein, family protein 7	1.83
MSMEG_1548	propanediol utilization: dehydratase, medium subunit	1.83
MSMEG_6011	short chain dehydrogenase	1.82
MSMEG_4850	short-chain dehydrogenase,reductase SDR	1.82
MSMEG_6618	nudix hydrolase	1.82
thiC	thiamine biosynthesis protein ThiC	1.80
MSMEG_3635	ironIII-transport system permease protein SfuB	1.80
MSMEG_0831	short chain dehydrogenase	1.79
MSMEG_4742	clavaldehyde dehydrogenase	1.79
MSMEG_6150	4-carboxymuconolactone decarboxylase	1.78
MSMEG_3298	response regulator receiver domain protein	1.76
MSMEG_2217	AMP-dependent synthetase and ligase	1.75
MSMEG_2719	hydrogen:quinone oxidoreductase	1.75
MSMEG_4042	transcriptional regulator, GntR family protein	1.74
MSMEG_6362	quinone oxidoreductase	1.72
MSMEG_4297	acyltransferase, ws,dgat,mgat subfamily protein	1.72
xth	exodeoxyribonuclease III, EC Number - 3.1.11.2	1.71
glnA	glutamine synthetase, catalytic domain, EC Number - 6.3.1.2	1.71
MSMEG_6630	glyoxalase family protein	1.71
MSMEG_4649	phytanoyl-CoA dioxygenase	1.70
dacB	D-alanyl-D-alanine carboxypeptidase,D-alanyl-D-alanine-endopeptidase, EC Number - 3.4.16.4	1.70
MSMEG_4251	cyclase,dehydrase	1.69
MSMEG_5372	sensor protein KdpD, EC Number - 2.7.13.3	1.68
MSMEG_5174	regulatory protein GntR, HTH	1.67
MSMEG_5331	SIS domain protein	1.67
aceA	isocitrate lyase, EC Number - 4.1.3.1	1.66
purC	phosphoribosylaminoimidazole-succinocarboxamide synthase, EC Number -	1.66
MSMEG_1838	cationic amino acid transporter	1.66
MSMEG_4146	quinone oxidoreductase	1.66
MSMEG_3995	N-carbamoyl-L-amino acid amidohydrolase	1.66

GeneName	Description	fold change
MSMEG_2606	ArsR-family protein transcriptional regulator	1.65
MSMEG_0533	gp236 protein	1.65
MSMEG_5405	transcriptional regulator, ArsR family protein	1.65
MSMEG_4396	isochorismatase hydrolase	1.64
MSMEG_2704	hydrogenase assembly chaperone HypC,HupF	1.64
MSMEG_0542	antar domain protein	1.64
MSMEG_5204	oxidoreductase, short chain dehydrogenase,reductase family protein	1.64
MSMEG_4362	universal stress protein family protein	1.64
wrbA	NADPH:quinone oxidoreductase, type IV, EC Number - 1.6.5.2	1.64
ackA	acetate kinase, EC Number - 2.7.2.1	1.64
MSMEG_3240	DNA-binding response regulator, LuxR family protein	1.63
MSMEG_4239	LppM protein	1.63
MSMEG_0051	transcription factor WhiB family protein	1.63
hutF	formiminoglutamate deiminase, EC Number - 3.5.3.13	1.62
MSMEG_3650	integral membrane protein	1.62
MSMEG_2760	polyphosphate glucokinase, EC Number - 2.7.1.63	1.62
MSMEG_3543	soluble secreted antigen MPT53	1.61
phoU	phosphate transport system regulatory protein PhoU	1.61
tpiA	triosephosphate isomerase, EC Number - 5.3.1.1	1.60
MSMEG_0544	transcriptional regulator	1.59
MSMEG_0786	serine,threonine protein kinase	1.59
MSMEG_5179	DoxX subfamily protein, putative	1.58
MSMEG_6235	thiopurine S-methyltransferase tpmt superfamily protein	1.58
MSMEG_5777	transcription regulator	1.56
MSMEG_2112	secreted protein	1.56
MSMEG_1878	S30AE family protein	1.56
MSMEG_1343	Trp tRNA	1.55
MSMEG_1205	cyclopropane-fatty-acyl-phospholipid synthase 1, EC Number - 2.1.1.79	1.55
cobL	precorrin-6Y C5,15-methyltransferase decarboxylating, EC Number - 2.1.1.132	1.54
MSMEG_5427	ribose-phosphate pyrophosphokinase, EC Number - 2.7.6.1	1.54
MSMEG_1919	Transcription factor WhiB	1.54
MSMEG_3425	TetR-family protein transcriptional regulator	1.53
MSMEG_6859	oxidoreductase	1.53
MSMEG_6892	replicative DNA helicase, EC Number - 3.6.1.- 3.1.-.-	1.53
MSMEG_6632	beta subunit of malonate decarboxylase	1.53
asnB	asparagine synthase glutamine-hydrolyzing, EC Number - 6.3.5.4	1.52
MSMEG_0266	arginine decarboxylase, EC Number - 4.1.1.19	1.52
MSMEG_0261	p40 protein	1.52
MSMEG_3357	metal-dependent phosphohydrolase, HD subdomain	1.52
68 Genes	Hypothetical proteins	1.53-

4.3.1.9 Description of important genes exclusively up and down regulated in DPI induced dormancy model

Iron repressor protein which is up-regulated is important for virulence of Mtb [36]. Also, ferric uptake regulator is up-regulated which is important for growth of many bacteria. NADH Ubiquinone oxidoreductase is up-regulated which shows importance of maintaining NADH pool during dormancy. Cytochrome P450, cytochrome P450 hydroxylase and cytochrome P450 monoxygenase are up-regulated. Cytochromes P450 (CYPs) are hemeproteins. They use various small and large molecules as substrates in enzymatic reactions. Usually, they work as terminal oxidase enzymes in ETC and are broadly categorized as P450-containing systems. Up-regulation of bi-functional wax ester synthase, acyl-CoA diacylglycerol acyltransferase indicates increased synthesis of Triacylglycerol (TAG) and wax ester under dormancy [37]. This also corroborates with the earlier findings obtained from multiple stress dormancy model.

Enoyl-CoA hydratase is down-regulated. It is an enzyme that hydrates the double bond between the second and third carbons on acyl-CoA. This enzyme is essential to metabolizing fatty acids to produce both acetyl CoA and energy. Deoxyribose phosphate aldolase is down-regulated which participates in pentose phosphate pathway by converting 2-deoxy D-ribose-5-phosphate to D-glyceraldehyde 3-phosphate and acetaldehyde (deoxyribose 5 phosphate). Down-regulation of the above genes and additionally acetyl-CoA synthetase as well as acetyl-CoA dehydrogenase shows that Energy metabolism pathways are down-regulated. Alcohol dehydrogenases are down-regulated. These enzymes which are expressed in many organisms play important role in the interconversion between alcohols and aldehydes or ketones with the reductionNAD^+ to NADH. Thus, NADH pool is maintained under dormant conditions.

Many genes encoding transcriptional regulators like LysR family protein; putative transcriptional regulatory protein; TetR-family protein transcriptional regulator; LysR-family protein transcriptional regulator; transcriptional regulator, LacI family protein; transcriptional regulator, GntR family protein; transcriptional regulator; AraC family protein are up-regulated. These proteins play important role in regulation of multidrug efflux pumps, biosynthesis of antibiotics and response to osmotic stress and toxic chemicals.Also participate in control of catabolic pathways,

differentiation processes, and pathogenicity. MerR-family protein transcriptional regulator is also up-regulated. The majority of MerR-family protein transcriptional regulators respond to environmental stimuli, such as oxidative stress, heavy metals or antibiotics [38]. IclR-family protein transcriptional regulator is up-regulated which comprises protein regulators having dual role as activayors as well as repressors. Glyoxylate shunt in Enterobacteriaceae is under regulation by a member of this family protein. Also, they are involved in contro of multidrug resistance, aromatics degradation, quorum-sensing signals inactivation and many more [39]. AsnC family protein; transcriptional regulator also known as feast/famine regulatory protein (FFRPs) is also up-regulated. They play important role in regulation of multiple cellular metabolisms such as amino acid metabolism, pili synthesis and also might be implicated in persistence [40].

Many transcriptional regulators like AsnC-family protein transcriptional regulator, MerR-family protein transcriptional regulator, IclR-family protein transcriptional regulator, TetR-family protein transcriptional regulator, GntR-family protein transcriptional regulator are down-regulated. Thus, the survival of bacteria under dormancy is highly organised.

Many ABC transporter genes are up-regulated which includes, ABC transporter permease protein; ABC transporter, ATP-binding protein GluA; ABC transporter efflux protein, DrrB family protein; ABC proline,glycine betaine family protein transporter, periplasmic ligand binding protein; ABC transporter, membrane spanning protein, putative; ABC heavy metal transporter, inner membrane subunit; sugar ABC transporter ATP-binding protein. The ATP Binding cassette proteins are expressed in a variety of organisms which protect the organism from toxic compounds [41]. The genes encoding sugar ABC transporter ATP-binding protein and ribose transport system permease protein RbsC are also up-regulated. They are involved in sugar catabolic operons in response to availability of sugars.

4.3.2 Global protein expression of active and dormant *M. smegmatis* bacilli

For global protein expression change we used a recent method, a new variant of label-free quantification known as LC/MS^E was introduced for quadrupole time-of-flight (Q-Tof) mass spectrometers. For this method, alternating scans of low collision

energy and elevated collision energy during LC/MS^E analysis are used to obtain both protein quantification and protein identification data in a single experiment [42-44]. The low-energy scan mode is used to obtain accurate precursor ion mass and intensity data for quantification, while the elevated collision energy mode generates multiplex peptide fragmentation of all peptide precursors with associated accurate mass product ion information for database searching and subsequent protein identification. In addition to relative quantification between samples based on electro-spray intensity, absolute quantification (moles protein) for each identified protein is possible using the LC/MS^E mode of acquisition [44]. This is predicated on the observation that average signal intensity measured by LC/MS^E of the three most intense tryptic peptides for any given protein is constant at a given concentration, regardless of protein size. In addition to tracking the relative changes in protein abundance in response to treatment, the ability to derive absolute quantities enables the stoichiometric comparison of proteins within and across samples. Moreover, LC/MS^E provides substantial advantages for protein identification over conventional LC/MS/MS approaches. Unlike data-dependent LC/MS/MS, where the most abundant precursors in an MS scan are sequentially subjected to MS/MS fragmentation, MS^E utilizes parallel, multiplex fragmentation where all peptide precursors are simultaneously fragmented throughout the chromatographic separation process regardless of intensity. This allows data-independent identification of lower abundance peptides and provides increased proteome coverage and dynamic range of protein identification compared to data-dependent LC/MS/MS.

4.3.2.1 Protein identification, validation and quantification.

After treatment, proteins were extracted and temporal changes in the proteome were characterized both qualitatively and quantitatively using LC/MS^E. The ion detection, clustering, and normalization were performed using ProteinLynx GlobalServer. Intensity measurements are typically adjusted on those components, *i.e.* deisotoped and charge state-reduced accurate mass retention time pairs, that replicate throughout the complete experiment for analysis at the accurate mass/retention cluster level. Components are typically clustered together with a <10 ppm mass precision and a <0.25-min time tolerance. Alignment of elevated energy ions with low energy

precursor peptide ions is conducted with an approximate precision of ±0.05 min. For analysis on the protein identification and quantification level the observed intensity measurements are normalized on the intensity measurement of the identified peptides of the digested internal standard. The observed intensity measurements were normalized for injection volume and protein load variability before conducting quantitative comparisons between conditions by applying scaling. A binary comparison of the peptide precursor intensity measurements of two injections of all three of the investigated conditions is discussed. A 45° diagonal line is obtained (Figure 4.7) with almost no variation throughout the detected range. This demonstrates the expected distribution in the instance of no obvious change between the investigated injections or conditions.

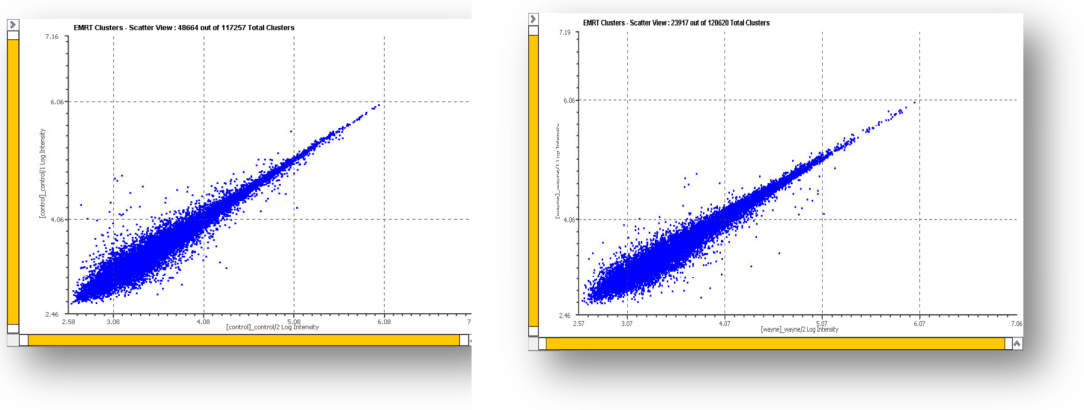
Proteins showing % coverage more than 30 were selected for analysis.

Table 4.8: Comparison of number of differentially regulated proteins in different dormant samples

Sample	**Up-regulated Proteins**	**Down-regulated Proteins**	**Unique to Wayne/DPI/Ascorbic acid**	**Unique to Control**
Wayne Model	107	221	71	61
DPI treated	397	54	99	50
Ascorbic acid treated	77	210	61	41

4.3.2.2 Statistical Validation of MS-MS Results.Scatter plots of peak intensity (on a logarithmic scale) within replicate data

(A) (B)



(C) (D)

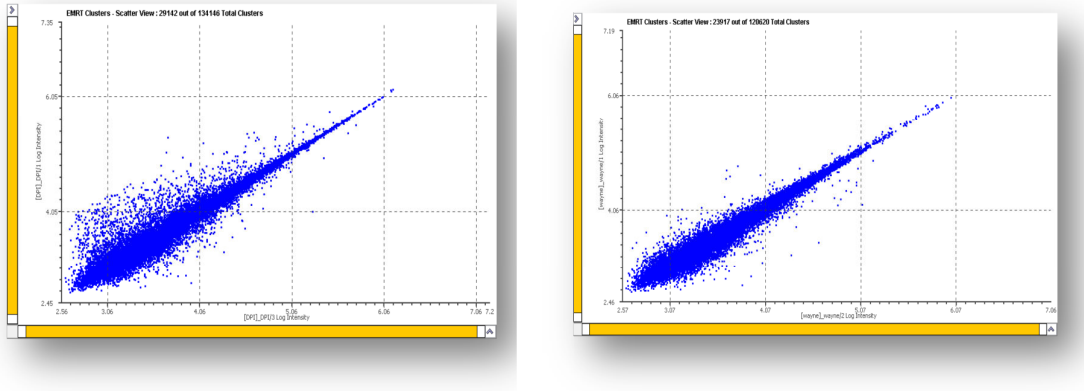
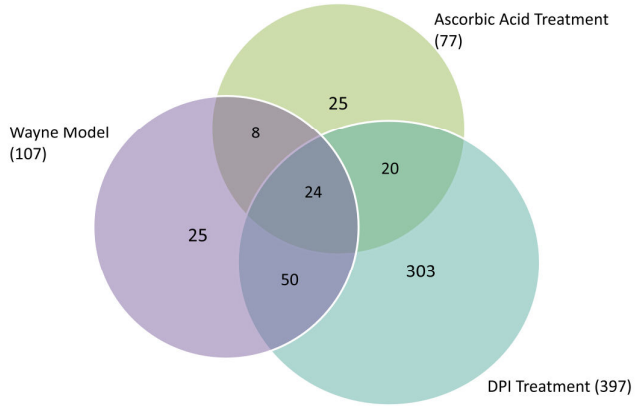


Figure 4.6: Scatter plots of peak intensity (on a logarithmic scale) within replicate data. (A) Aerobic sample (control), (B) Wayne Model culture, (C) DPI treated sample and (D) Ascorbic acid treated sample.

4.3.2.3 Diagrammatic representation of differentially expressed proteins under three different conditions

(A)



(B)

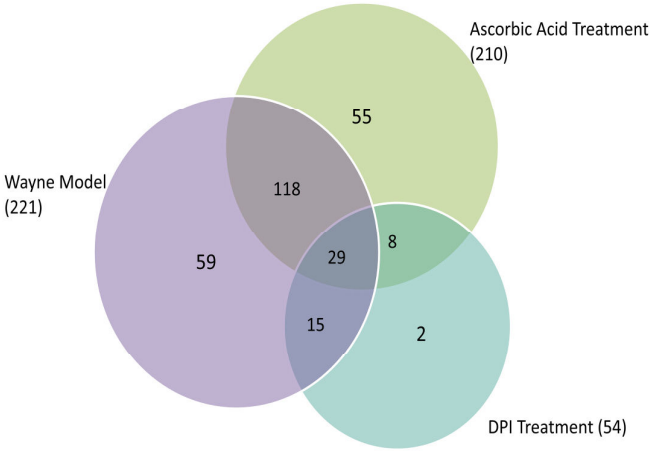


Figure 4.7: Venn diagramme of proteins differentially expressed under three different conditions. (A) Diagrammatic representation of up-regulated proteins under 3 different conditions. (B) Diagrammatic representation of down-regulated proteins under 3 different conditions.

4.3.2.4 List of common proteins up or down regulated under all three conditions

Table 4.9: List of common proteins up-regulated under all three conditions

Accession Number	Description	Fold Change				
		AA	DPI	Wayne	Mass (Daltons)	PI (PH)
A0QNF5	Cell wall synthesis protein CwsA OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN cwsA	Detected in all three	Detected in all three	Detected in all three	24,905	6.
A0QP01	O methyltransferase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 0224 PE 4 SV	Detected in all three	Detected in all three	Detected in all three	23,130	5.4
A0QQQ1	Superoxide dismutase Cu Zn OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN sodC PE 3	Detected in all three	Detected in all three	Detected in all three	23,207	4.5
A0QR19	Uroporphyrinogen III synthase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN hemD PE	Detected in all three	Detected in all three	Detected in all three	63,808	5.5
A0QSD2	50S ribosomal protein L4 OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN rplD PE 1 SV	Detected in all three	Detected in all three	Detected in all three	22,872	12.1
A0QSD8	50S ribosomal protein L16 OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN rplP PE 1 SV	Detected in all three	Detected in all three	Detected in all three	15,729	11.9
A0QSP0	Coenzyme B12 dependent glycerol dehydrogenase small subunit OS Mycobacterium smegmatis strain ATCC	Detected in all three	Detected in all three	Detected in all three	15,415	5.2
A0QWQ4	Alanine tRNA ligase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN alaS PE 1 SV 1	Detected in all three	Detected in all three	Detected in all three	96,770	5
A0QYE3	ComA operon protein 2 OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 3628 PE 4	Detected in all three	Detected in all three	Detected in all three	13,937	5.1
A0QZ83	14 kDa antigen OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN hspX PE 3 SV 1	Detected in all three	Detected in all three	Detected in all three	15,919	5.3
A0R051	Ubiquinol cytochrome c reductase iron sulfur subunit OS Mycobacterium smegmatis strain ATCC 700084	Detected in all three	Detected in all three	Detected in all three	44,810	7.5
A0R0Q9	Acyl CoA oxidase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 4474 PE 4 SV 1	Detected in all three	Detected in all three	Detected in all three	70,482	9
A0R1Z7	ATP cob I alamin adenosyltransferase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN M	Detected in all three	Detected in all three	Detected in all three	20,828	8.8
A0R3D6	Putative ligase MSMEG 5435 MSMEI 5285 OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN	Detected in all three	Detected in all three	Detected in all three	58,778	8.2
A0R4H0	29 kDa antigen CFP29 OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN cfp29 PE 4 SV 1	Detected in all three	Detected in all three	Detected in all three	28,730	4.6
A0R5R5	Cyclopropane fatty acyl phospholipid synthase OS Mycobacterium smegmatis strain ATCC 700084 mc 2	Detected in all three	Detected in all three	Detected in all three	48,639	9.6
P48354	DNA gyrase subunit A OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN gyrA PE 3 SV 1	Detected in all three	Detected in all three	Detected in all three	93,175	5.6
Q9ZHC5	DNA binding protein HU homolog OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN hup PE	Detected in all three	Detected in all three	Detected in all three	21,222	12.4

Table 4.10: List of common proteins down-regulated under all three conditions

Accession Number	Description	Fold Change				
		DPI	AA	Wayne	Mass (Daltons)	PI (PH)
A0QPJ7	PEP phosphonomutase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 0422 PE 4 SV	Unique to control	Unique to control	Unique to control	26,685	4.4
A0QQ71	Phosphate import protein PhnD OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN phnD PE	Unique to control	Unique to control	Unique to control	33,377	6.1
A0QQA8	Aspartate aminotransferase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 0688	3.0	2.3	1.6	47,181	5.5
A0QRP7	TROVE domain protein OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 1193 PE 4 S	Unique to control	Unique to control	Unique to control	61,175	9.2
A0QS15	Secreted protein OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 1313 PE 4 SV 1	Unique to control	Unique to control	Unique to control	82,342	5.2
A0QSB4	Uncharacterized protein OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 1419 PE	Unique to control	Unique to control	Unique to control	25,684	11
A0QU18	Monoxygenase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 2045 PE 4 SV 1	2.0	3.4	1.9	49,537	5.4
A0QWQ9	Chorismate synthase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN aroC PE 3 SV 1	1.5	4.2	2.5	41,938	6.8
A0QX75	Quinolinate synthetase complex A subunit OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155	Unique to control	Unique to control	Unique to control	36,854	5.4
A0QX81	Histidinol dehydrogenase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN hisD PE 3 SV	Unique to control	Unique to control	Unique to control	46,697	4.7
A0QZ23	Alpha ketoglutarate dependent taurine dioxygenase OS Mycobacterium smegmatis strain ATCC 700084 m	Unique to control	Unique to control	Unique to control	33,101	5.8
A0QZM8	Putative acyl CoA dehydrogenase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG	Unique to control	Unique to control	Unique to control	41,805	6.2
A0QZM9	Nitrilotriacetate monooxygenase component A OS Mycobacterium smegmatis strain ATCC 700084 mc 2 15	Unique to control	Unique to control	Unique to control	46,781	6.5
A0QZX2	Phosphoribosyl ATP pyrophosphatase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN his	Unique to control	Unique to control	Unique to control	10,209	5.1
A0R0I8	Phosphonoacetaldehyde hydrolase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG	3.2	4.2	2.8	25,104	4.5
A0R120	Rhodanese domain protein cystathionine beta lyase OS Mycobacterium smegmatis strain ATCC 700084 m	Unique to control	Unique to control	Unique to control	55,190	6.2
A0R3K8	Alkaline phosphatase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 5508 PE 4 S	Unique to control	Unique to control	Unique to control	57,429	4.6
A0R3V8	Amino acid acetyltransferase OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 561	Unique to control	Unique to control	Unique to control	22,075	5.5

Accession Number	Description	Fold Change				
		DPI	AA	Wayne	Mass (Daltons)	PI (PH)
A0R3Y1	Uncharacterized protein OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN MSMEG 5635 PE	Unique to control	Unique to control	Unique to control	6,860	10
A0R462	Pyridoxamine 5 phosphate oxidase family protein OS Mycobacterium smegmatis strain ATCC 700084 mc	Unique to control	Unique to control	Unique to control	15,865	8.4
A0R4C0	Phosphate import ATP binding protein PstB OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155	Unique to control	Unique to control	Unique to control	28,093	4.3
Q9F868	Sensory transduction protein regX3 OS Mycobacterium smegmatis strain ATCC 700084 mc 2 155 GN reg	1.7	3.4	1.9	24,903	4.5

4.3.2.5 List of pathways differentially regulated in DPI treated sample

Total 70 pathways were identified in Kegg pathway database using proteins down regulated in DPI treated sample whereas total 60 pathways were identified in Kegg pathway database using proteins up regulated in DPI treated sample. Some of the important pathways are enlisted in Table 4.17.

Table 4.11: List of pathways obtained from kegg pathway database which includes differentially expressed in DPI treated sample

GO_id	Pathway	Number of proteins down regulated in DPI	Number of proteins up regulated in DPI	P-value
msm01100	Metabolic pathways	73	48	9.02E-01
msm03010	Ribosome	35	0	6.98E-24
msm03020	RNA polymerase	3	0	2.78E-03
msm00970	Aminoacyl-tRNA biosynthesis	7	2	1.27E-02
msm03030	DNA replication	1	0	1.00E+00
msm00280	Valine, leucine and isoleucine degradation	13	4	6.35E-02
msm00260	Glycine, serine and threonine metabolism	6	4	1.64E-01
msm00230	Purine metabolism	10	6	2.19E-01
msm00240	Pyrimidine metabolism	6	1	3.03E-01
msm00400	Phenylalanine, tyrosine and tryptophan biosynthesis	2	4	7.23E-01
msm00250	Alanine, aspartate and glutamate metabolism	2	2	9.34E-01
msm00340	Histidine metabolism	2	3	9.34E-01
msm00330	Arginine and proline metabolism	1	3	1.00E+00

msm00300	Lysine biosynthesis	1	4	1.00E+00
msm00730	Thiamine metabolism	1	0	1.00E+00

4.3.2.6 Global expression change in DPI treated dormant sample

Analysis of three independent experiments using PLGS V 2.2.5 software revealed significant change in the level of expression of proteins.

Metabolic Pathways

73 proteins are down-regulated and 48 proteins are up-regulated from metabolic pathways in DPI treated sample.Difference in the number indicates overall shut down of metabolic processes. The succinate dehydrogenase subunit A (sdhA) is down-regulated. It participates in TCA cycle and respiratory chain. Inosine 5'-monophosphate dehydrogenase is down regulated which is a purine biosynthetic enzyme that catalyzes the nicotinamide adenine dinucleotide (NAD^+)-dependent oxidation of inosine monophosphate (IMP) to xanthosine monophosphate (XMP), the first committed and rate-limiting step towards the *de novo* biosynthesis of guanine nucleotides from IMP. IMPDH is a regulator of the intracelluar guanine nucleotide pool, and is therefore important for DNA and RNA synthesis, signal transduction, energy transfer, glycoproteinsynthesis, as well as other process that are involved in cellular proliferation. Proteins like EPTC-inducible aldehyde dehydrogenase, enoyl-CoA hydratase, Fructose 1,6-Bisphosphatase, S-adenosylmethionine synthase (metk) are all down-regulated in DPI treated sample. MetK has been shown to be involved in growth of *E. coli* [45]. TrpD which is involved in tryptophan biosynthesis is necessary for growth of *M. tuberculosis* [46]. As many proteins are down regulated, on the other hand some selective proteins are up regulated which might play important role in dormancy. And therefore, it is important to study these proteins. Proteins like isocitrate dehydrogenase, succinyl-CoA synthetase subunit alpha, fructose-1,6-bisphosphate aldolase, Adenosine kinase, Glutamine synthetase, isocitrate lyase, Malate synthase G, thiosulfate sulfurtransferase, Acetaldehyde dehydrogenase, 3-isopropylmalate dehydrogenase, uroporphyrin-III C-methyltransferase are all up regulated in DPI treated sample. Isocitrate lyase (icl) and malate synthase are highly used by bacteria, fungi and plants. It is the central enzyme in glyoxylate shunt pathway in which the two decarboxylation steps of the tricarboxylic acid cycle (TCA cycle) are

bypassed.[47]. Both the enzymes are shown to be highly up regulated in other known dormancy models like Non-culturable dormancy model in *M. smegmatis* and also in hypoxic conditions [17, 18]. Transcriptional regulator IcIR family protein which regulates the glyoxylate bypass operon genes is also up-regulated.

Chaperones

The non-covalent folding or unfolding of proteins and the assembly or disassembly of other macromolecular structures is regulated by chaperones.Proteins like Universal stress protein family protein (A0QYW6), Probable cold shock protein A (cspA A0R5E1), 10 kDa chaperonin (groS A0QSS3) are up regulated. These proteins are expressed in hypoxic conditions. The 60 kDa chaperonin 1 (A0QQU5) is stress-induced, acting to stabilise or protect disassembled polypeptides under heat-shock conditions. Whereas, 60 kDa chaperonin 2 protects cell from ROS, upregulated in response to H_2O_2[48].

Methyltransferases

Expression of methyl transferase is very low in vitro and non-stressed conditions but strongly up-regulated in vivo and stressed condition. This up-regulation varies from 100-150 folds under different stress condition. We identified three methyltransferases up regulated in DPI treated sample namely, Putative O methyltransferase (A0R2D5), O methyltransferase (A0QP01) and Sulfurtransferase (sseA A0QTE3) which is induced in drug resistant dormancy.

Transcriptional Factors

Transcription factors bind to specific DNA sequences and control the rate of transcription [49]. Transcription factors execute this function without help or sometimes with other proteins in a complex [50]. We identified up regulation of Transcriptional regulator TetR family protein (A0QQS3) which is involved in biosynthesis of antibiotics, efflux pumps (tetracyclin resistance), LacI family protein transcriptional regulator (A0QWL9) which is important for carbon source utilization, Transcriptional regulator IclR family (A0QXK4) that regulates glyoxylate

bypassoperon genes. Some more regulators like Iron dependent repressor IdeR (A0QVZ3) and Probable transcriptional regulatory protein (A0QWH1) are also up regulated.

Ribosome, RNA, DNA and Amino Acids

35 out of 35 proteins in Ribosome are down regulated whereas all proteins in RNA polymerase are down regulated. 7 of 9 proteins from amino acyl t-RNA biosynthesis are down regulated. No protein involved in DNA replication is up regulated. Purine and pyrimidine biosynthesis is highly repressed. Thus, this indicates that the RNA the replication assembly as well as ribosome assembly is highly repressed. Metabolism of amino acids like alanine, aspartate, glutamate, arginine, lysine, proline, thiamine, and histidine is also down regulated.

4.3.2.7 Comparison of fold change between at transcriptional and translational level of selected proteins

Table 4.12: Proteins down-regulated at both transcriptional and translational level

Accession Number	Description	Gene Name	Fold change at gene level			Fold change at protein level		
			Wayne	DPI	AA	Wayne	DPI	AA
A0QZM9	nitrilotriacetate monooxygenase component A, EC Number - 1.14.13.	MSMEG_4085	33.3	95.9	14.0	Unique to control	Unique to control	Unique to control
A0R120	rhodanese domain protein,cystathionine beta-lyase	MSMEG_4589	12.5	13.5	9.1	Unique to control	Unique to control	Unique to control
A0R4C0	phosphate ABC transporter, ATP-binding protein, EC Number - 3.6.3.27	pstB	8.0	7.5	7.7	Unique to control	Unique to control	Unique to control
Q9F868	DNA-binding response regulator RegX3	regX3	6.1	6.9	6.0	1.7	3.4	1.9
A0QU18	monoxygenase	MSMEG_2045	16.5	15.0	10.4	2	3.4	1.9

4.4 Discussion

In the previous chapters we have seen that *M. smegmatis* actively produces superoxide. The major source of this superoxide is membrane bound NADH oxidase. We have also seen that by blocking superoxide production with NADH oxidase inhibitor, *M. smegmatis* goes into dormancy. We have also characterised this dormancy using different standard parameters like acid fastness, size determination, Dos Regulon expression, antibiotic resistance and CFU determination. And thus, we have developed a new dormancy model which can be used to study the dormancy as well as screen new anti-tubercular drugs. To develop new drugs, identification of targets should be done for which the precise nature of the dormant forms should be known. The molecular mechanism which makes the dormant form different than the active form would help us to develop new targets for drug development. Therefore, to study the molecular mechanisms, we studied the global change in gene expression as well as protein expression in DPI induced dormant *M. smegmatis*. To validate our results with known dormancy models, we have also used Wayne's dormancy model along with ascorbic acid induced dormancy model. We hypothesize that ascorbic acid induced dormancy model follows a similar mechanism like DPI to achieve dormancy. All these three models are compared with aerobically growing *M. smegmatis* bacilli which served as a control.

The purpose of this study was to see the common genes and proteins which are up/down regulated under all three conditions and thus, validate our DPI induced dormancy model and find out new genes/proteins which are exclusively up regulated in our DPI induced dormancy model which could be used as targets for drug development with further studies. We found total 1204 genes up-regulated and 1501 genes were down-regulated in Wayne hypoxia model. In DPI induced dormancy model, 1499 genes were up-regulated and 1736 genes were down-regulated. In ascorbic acid induced dormancy model, 1019 genes were up-regulated and 1451 genes were down-regulated. As in all three models, the number of down-regulated genes is more than the respective up-regulated genes, shows that in dormancy, maximum number of cell processes are shut down barring a few which are necessary for maintenance and survival. Under all three conditions 762 genes were found to be up-regulated and 1362 were down-regulated. Thus, higher number of common genes which were up/down regulated indicates similarity between different dormancy

models. In LC-MS^E studies, total 397 proteins were found to be up-regulated and 54 proteins were found to be down-regulated in DPI induced dormancy model. In Wayne model, 107 proteins were found to be up-regulated and 221 proteins were found to be down-regulated. In ascorbic acid induced dormancy model, 77 proteins were found to be up-regulated and 210 proteins were found to be down-regulated. 24 proteins were found to be up-regulated and 29 were down-regulated under all three conditions. We identified 367 genes and 303 proteins which were up-regulated exclusively in DPI induced dormancy model. Further study of this is in progress which will come up with targets against the dormant form of mycobacteria which is the need of the hour.

There are some common proteins which are down-regulated at both, transcriptional as well as translational level; although a big number of microarray and proteomic data is not matching with each other. One possible reason could be that, the regulation at both the levels is different in cell and therefore such a difference could be then observed. Some of the common proteins which are down-regulated under all three conditions at both transcriptional as well as translational levels are given in the table (Table 4.11).

A decrease in ATP level during dormancy leads to cessation of ribosomal as well as other general protein synthesis. We found down-regulation of genes for different ATP synthase subunits namely, atpA, atpB, atpC, atpD, atpE, atpG and cydB under all three conditions. Down-regulation of cytocrome c reductase is observed in all three models (8 fold, 7 fold and 561 fold in Wayne, DPI and AA model respectively). It is required for ATP generation. This shows the importance of ATP synthesis in active growth whereas its requirement drastically reduced during dormancy. Many enzymes like cytocrome c reductase are down-regulated to a greater extent in AA induced dormancy than in other two models. Thus, ascorbic acid could be having multiple mechanisms of inducing dormancy because it a non specific inhibitor of ROS.

As reported earlier, we found a similar shift in metabolism from the available set of genes and proteins when compared between active and dormant state [51-55]. The up-regulated genes involved in lipid metabolism during dormancy (Table 4.2) confirmed the previous evidence about the involvement of lipids in virulence of Mtb [56, 57]. In dormant cells we found a shift in carbohydrate metabolism where TCA

cycle reroutes towards glyoxylate cycle as described in earlier reports [55]. 15 of 17 genes in TCA cycle are down regulated indicating general slowdown of metabolism. Transcriptional regulator IclR family protein (A0QXK4) that regulates glyoxylate bypassoperon genes was found to be up regulated.We found a strong up-regulation of isocitrate lyase, the gate enzyme of the glyoxylate shunt [19-22] and malate synthase proteins in all three dormancy models whereas the genes encoding isocitrate lyase and malate synthase were down regulated in all three models. Thus, further validation of microarray data should be done to solve the ambiguity. Malate can be used to generate pyruvate and further to replenish the pool of glycolytic intermediates by gluconeogenesis. Many dehydrogenases like alanine dehydrogenase, alcohol dehydrogenase, aldehyde dehydrogenase and oxovalerate dehydrogenase are up-regulated. This could be required to maintain the NADH pool under dormant conditions. Alanine dehydrogenase has been well documented in dormancy in Mtb and *M. smegmatis* and is identified as one of the virulence factors secreted by pathogenic Mtb [14, 15].

Metabolism of amino acids like alanine, aspartate, glutamate, arginine, lysine, proline, thiamine, and histidine is also down regulated at transcriptional as well as translational level. This shows that overall the protein synthesis is down regulated in dormancy. The 60 kDa chaperonin 1 (A0QQU5) is up regulated at translational level. It is stress-induced, acting to stabilise or protect disassembled polypeptides under heat-shock conditions. Whereas, 60 kDa chaperonin 2 protects cell from ROS, upregulated in response to H_2O_2. Genes encoding proteins such as ABC type drug export system membrane protein, ABC type drug export system ATP binding protein, bacterial extracellular solute binding protein, sugar ABC transporters are all up-regulated. All these results show that the bacilli possess a well equipped machinery to survive under stressed conditions.

DNA replication is highly down regulated at both gene and protein level. Similarly, genes like UDP-N-acetylmuramoylalanine-D-glutamate ligase (murD), phospho-N-acetylmuramoyl-pentapeptide-transferase (mraY), UDP-N-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase involved in peptidoglycan synthesis are also down regulated. Genes involved in ribosome are also down regulated. Thus, it is very clear that the cell is investing minimum energy in processes like replication, cell division, transcription, translation etc.

Up regulation of gene encoding iron suppressor protein was seen only in DPI dormancy model. This protein is essential for virulence of Mtb. As well as, gene encoding Ferric uptake regulator which plays crucial role in growth of many organisms was seen to be up regulated only in DPI dormancy model

Many genes encoding transcriptional regulators like LysR family protein, IcIR family protein, TetR family protein, GntR family protein, AraC family protein are up regulated only in DPI induced dormancy model. These proteins are involved in many cellular functions like control of multidrug efflux pumps, pathways for the biosynthesis of antibiotics, glyoxylate shunt, and response to osmotic stress, environmental stimuli, oxidative stress and toxic chemicals, control of catabolic pathways, differentiation processes, and pathogenicity. Thus, these factors could lead us to understand the signalling processes that are necessary for survival under stress conditions.

Many genes encoding redox sensitive proteins were found to be down-regulated namely thioredoxin, thioredoxin reductase, thioredoxin-disulfide reductase, phosphoglycerate kinase, 4Fe-4S ferredoxin, iron-sulfur binding protein, alkylhydroperoxide reductase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), type I and tRNA adenine-N1-methyltransferase.Thioredoxin superfamily of proteins mediate cytoplasmic protein reduction via generalized thiol/disulfide exchange reactions and also maintenance of cellular redox homeostasis [58]. Thioredoxin reductase is shown to be involved in antioxidant defence [59]. GAPDH and phosphoglycerate kinase have been shown as oxidation sensitive proteins identified with OxICAT which are involved in carbon metabolism. Redox regulation of GAPDH is mainly by S-glutathionylation of itsactive site cysteine Cys150 [60]. Ferrodoxins are iron-sulfur proteins that participate in electron exchange reactions and have been shown to be essential for growth of the bacterium, *Pseudomonas aeruginosa*[61]. The alkylhydroperoxide reductase is widely studied in mycobacterium tuberculosis. It has three cysteine residues; all are required for activity [62]. Redox sensitive proteins play important role in signal transduction mechanisms. Therefore, it is very important to study which proteins are modulated by ROS and how they affect the cellular processes. Thus, identifying proteins having cysteine residues as well as sulfur residues may help in identifying redox sensitive targets. Further extension of our

study will be to examine the redox sensitive proteins and their role in signalling and cellular mechanisms.

This report mainly helps in understanding the metabolic shift during dormancy. Further, we have identified several genes/proteins that were unique/abundant in DPI induced dormancy model such as many transporters, transcriptional regulators etc. Their role in survival of the dormant stage needs further research. This study also supports the identification of potential drug targets against latent Tuberculosis which is the need of the hour.

4.4 References

1. Betts J.C., Lukey P.T., Robb L.C., McAdam R.A. and Duncan K., Evaluation of a nutrient starvationmodel of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling, Molecular Microbiology, 43: 717–731, 2002.

2. O'Brien R.J., and Nunn P.P., The need for new drugs against tuberculosis-obstacles, opportunities and next steps, Am J Resp Crit Care Med,163**:** 1055–1058, 2001.

3. Filippini P., Iona E., Piccaro G., Peyron P., Neyrolles O. and Fattorini L., Activity of Drug Combinations against Dormant *Mycobacterium tuberculosis,* Antimicrobial Agents And Chemotherapy, 54:2712-2715, 2010.

4. Dick T., Lee B. G. and Murugasu-Oei B., Oxygen depletion induced dormancy in *Mycobacterium smegmatis*, FEMS Microbiology Letters, 163:159-164.

5. Taneja N.K., Dhingra S., Mittal A., Naresh M. and Tyagi J.S., *Mycobacterium tuberculosis* Transcriptional Adaptation, Growth Arrest and Dormancy Phenotype Development Is Triggered by Vitamin C, PLoS ONE, 5:e10860, 2010.

6. Boon C., Li R., Qi R. and Dick T., Proteins of *Mycobacterium bovis BCG* induced in the Wayne dormancy model. J Bacteriol, 183:2672–2676, 2001.

7. Jungblut P. R., Schaible U. E., Mollenkopf H.J. et al, Comparative proteome analysis of *Mycobacterium tuberculosis*and *Mycobacterium bovis BCG* strains: towards functional genomicsof microbial pathogens, Mol Microbiol, 33:1103–1117, 1999.

8. Mattow J., Jungblut P.R., Muller E.C. and Kaufmann S.H., Identification of acidic, low molecular mass proteins of *Mycobacterium tuberculosis* strain H37Rv by matrix-assisted laserdesorption/ionization and electrospray ionization mass spectrometry, Proteomics, 1:494-507, 2001.

9. Monahan I.M., Betts J., Banerjee D.K. and Butcher P.D., Differential expression of mycobacterial proteins following phagocytosisby macrophages, Microbiology, 147:459–471, 2001.

10. Rosenkrands I., King A., Weldingh K., Moniatte M., Moertz E. and Andersen P., Towards the proteome of *Mycobacterium tuberculosis*, Electrophoresis, 21:3740–3756, 2000.

11. Sonnenberg M.G. and Belisle J.T., Definition of *Mycobacterium tuberculosis* culture filtrate proteins by two-dimensionalpolyacrylamide gel electrophoresis, N-terminal amino acid sequencing and electrospray mass spectrometry, Infect Immun., 65:4515-4524, 1997.

12. Wong D.K., Lee B.Y., Horwitz M.A. and Gibson B.W., Identification of fur, aconitase, and other proteins expressed by*Mycobacterium tuberculosis* under conditions of low and highconcentrations of iron by combined two-dimensional gel electrophoresisand mass spectrometry, Infect Immun., 67, 327–336, 1999.

13. Yuan Y., Crane D.D. and Barry C.E. 3rd, Stationaryphase-associated protein expression in *Mycobacterium tuberculosis*: function of the mycobacterial alpha-crystallin homolog, J Bacteriol., 178:4484-4492, 1996.

14. Harboe M., The significance of proteins actively secreted by*Mycobacterium tuberculosis* in relation to immunity and complications of mycobacterial diseases, Int J Lepr Other Mycobact Dis., 60:470–476, 1992.

15. Hutter B. and Dick T., Increased alanine dehydrogenase activityduring dormancy in *Mycobacterium smegmatis*, FEMS Microbiol Lett., 167:7–11, 1998.

16. Gupta S., Pandit S.B., Shrinivasan N., Chatterji D., Proteomics analysis of carbon-starved *Mycobacterium smegmatis*: induction of Dps-like protein, Protein Engineering, 15:503-511, 2002.

17. Salina E.G., Zhogina Y.A., Shleeva M.O., Sorokoumova G.M., Selishcheva A.A. and Kapreylants A.S., Biochemical and Morphological Changes in Dormant ("Nonculturable") *Mycobacterium smegmatis* Cells, Biochemistry (Moscow), 75:72-80, 2010.

18. Watanabe S., Zimmermann M., Goodwin M.B., Sauer U., Barry C.E.3rd and Boshoff H.I., Fumarate Reductase Activity Maintains an Energized Membrane in Anaerobic *Mycobacterium tuberculosis*, PLoS Pathogenesis, 7:e1002287, 2011.

19. Kumar R., Glyoxylate shunt: Combating Mycobacterium at forefront, International Journal of Integrative Biology, 7:69-72, 2009.

20. Graham J.E. and Clark Curtiss J.E., Identification of *Mycobacterium tuberculosis* RNAs synthesised in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS), PNAS USA, 96:11554-9, 1999.

21. McKinney J.D., Honer zu Bentrup K., et al, persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase, Nature, 406:735-8, 2000.

22. Lorenz M.C. and Fink G.R., The glyoxylate cycle is required for fungal virulence, Nature, 412:83-6, 2001.

23. Cheng F, Blackburn K, Lin Y, Goshe MB, Williamson JD. 2009. Absolute Protein Quantification by LC/MSE for Global Analysis of Salicylic Acid-Induced Plant Protein Secretion responses,Journal of Proteome Research*,*8*,* 82–93.

24. Kumar R. and Sanyal S., Mycobacterium tuberculosis: Dormancy, Persistence and Survival in the Light of Protein Synthesis, Understanding Tuberculosis - Deciphering the Secret Life of the Bacilli, Dr. Pere-Joan Cardona (Ed.), ISBN: 978-953-307-946-2, InTech, Available from: http://www.intechopen.com/books/understanding-tuberculosis-deciphering-the-secret-life-of-thebacilli/mycobacterium-tuberculosis-dormancy-persistence-and-survival-in-the-light-of-protein-synthesis, 2012.

25. Andries K., Verhasselt P., Guillemont J. et al., A diarylquinoline drug active on the ATP synthesis of *Mycobacterium tuberculosis*, Science*,* 307:223-227,2005.

26. Boyer P.D., A research journey with ATP synthase, J Biol Chem, 277:39045-39061, 2002.

27. Sassetti C.M. and Rubin E.J., The open book of infectious diseases, Nat Med., 13**:**279-280, 2007.

28. Khan A. and Sarkar D., Identification of a respiratory-type nitrate reductase and its role for survival of *Mycobacterium smegmatis* in Wayne model,Microbial Pathogenesis,
41:90-95, 2006.

29. Khan A., Akhtar S., Ahmad J.N. and Sarkar D., Presence of a functional nitrate assimilation pathway in *Mycobacterium smegmatis,* Microbial Pathogenesis 44:71-77, 2008.

30. Wayne L.G. and Sohaskey C.D., Nonreplicating persistence of Mycobacterium tuberculosis, Annu. Rev. Microbiol, 55:139-163, 2001.

31. Cusack S., Structures of Two Bacterial Prolyl-tRNA Synthetases with and without a cis-Editing Domain, Structure, 14:1511-1525, 2006.

32. Doublie S., Bricogne G., Gilmore C. and Carter C.W., Tryptophanyl-tRNA synthetase crystal structure reveals an unexpected homology to tyrosyl-tRNA synthetase, Structure, 3:17-31, 1995.

33. Primm T.P., Andersan S.J., Mizrahi V., Avarbock D., Rubin H. and Barry III C.E., The Stringent Response of *Mycobacterium tuberculosis* Is Required for Long-Term Survival, Journal of Bacteriology, 182:4889-4898, 2000.

34. Yao L., Cukier R.I. and Yan H., Catalytic Mechanism of Guanine Deaminase: An ONIOM and Molecular Dynamics Study, J. Phys. Chem., 111:4200-4210, 2007.

35. Berney M. and Cook G.M., Unique Flexibility in Energy Metabolism Allows Mycobacteria to Combat Starvation and Hypoxia, PLoS ONE, 5:e8614, 2010.

36. Manabe Y.C., Saviola B.J., Sun L., Murphy J.R. and Bishai W.R., Attenuation of virulence in *Mycobacterium tuberculosis* expressing a constitutively active iron repressor, PNAS, 96:12844-12848, 1999.

37. Deb C., Lee C., Dubey V.S., Daniel J., Abomoelak B., Sirakova T.D., Pawar S., Rogers L. and Kolattukudy P.E., A novel *in vitro* multiple-stress dormancy model for *Mycobacterium tuberculosis* generates a lipid-loaded, drug-tolerant, dormant pathogen, PLoS ONE, 4:1-15, 2009.

38. Brown N.L., Stoyanov J.V., Kidd S.P. and Hobbman J.L., The MerR family of transcriptional regulators, FEMS Microbiology Reviews, 27:145-163, 2005.

39. Molina-Henares A.J., Krell T., Guazzaroni M.E., Segura A. and Ramos J.L., Membersof theIclRfamilyof bacterial transcriptional regulators functionas activatorsand/or repressors, FEMS Microbiol Rev., 30:157–186, 2006.

40. Deng W., Wang H. and Xie J., Regulatory and Pathogenesis Roles of Mycobacterium Lrp/AsnC Family Transcriptional Factors, Journal of Cellular Biochemistry, 112:2655–2662, 2011.

41. Couture L., Nash J.A. and Turgeon J., The ATP-Binding Cassette Transporters and Their Implication in Drug Disposition: A Special Look at the Heart, Pharmacological Reviews, 58:244-258, 2006.

42. Chakraborty A.B., Berger S.J. and Gebler J.C., Use of an integrated MS: multiplexed MS/MS data acquisition strategy for high coverage peptide mapping studies, Rapid Commun. Mass Spectrom., 21:730–744, 2007.

43. Silva J., Denny R., Dorschel C., Gorenstein M., Kass I., Li Z., McKenna T., Nold M., Richardson K., Young P. and Geromanos S.J.,Quantitative proteomic analysis by accurate mass retention time pairs, Anal. Chem., 77:2187–2200, 2005.

44. Silva J.C., Denny R., Dorschel C.A., Gorenstein M., Li Z., Richardson K., Wall D., Geromanos S.J., Simultaneous qualitative and quantitative analysis of the *Escherichia coli* proteome: a sweet tale, Mol. Cell. Proteomics, 5:589–607, 2006.

45. Wei H. and Newman E.B., Studies on the role of the *metK* gene product of *Escherichia coli* K-12, Molecular Microbiology, 43:1651–1656, 2002.

46. Smith D.A., Parish T., Stoker N.G. and Bancroft G.J., Characterization of Auxotrophic Mutants of *Mycobacterium tuberculosis* and Their Potential as Vaccine Candidates, Infection and Immunity, 69:1142-1150, 2001.

47. Dunn M.F., Trujillo J.A.R. and Hernandez-Lucas I., Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis, Microbiology, 155:3166–3175, 2009.

48. Richardson R.T. and Alekseev O.M., Nuclear Autoantigenic Sperm Protein (NASP), a Linker Histone Chaperone That Is Required for Cell Proliferation,J. Biol. Chem., 281:21526-21534, 2006.

49. Latchaman D.S., Transcription factors: An overview, The International Journal of Biochemistry & Cell Biology, 29:1305-1312, 1997.

50. Lee T.I. and Young R.A., Transcription of eukaryotic Protein-coding genes, Annu. Rev. Genet., 34:77–137, 2000.

51. Boyer P.D., A research journey with ATP synthase, J Biol Chem, 277:39045-39061, 2002.

52. Sassetti C.M. and Rubin E.J., The open book of infectious diseases, Nat Med 13**,** 279-280, 2007.

53. Shi L., Jung Y.J., Tyagi S., Gennaro M.L. and North R.J., Expression of Th1-mediated immunity in mouse lung induces a *Mycobacterium tuberculosis* transcription pattern characteristic of non replicating persistence, PNAS, 100:241-246, 2003.

54. Shi L., Sohaskey C.D., Kana B.D., Dawes S., North R.J., Mizrahi V. and Gennaro M.L., Changes in energy metabolism of *Mycobacterium tuberculosis*in mouse lung and under *in vitro* conditions affecting aerobic respiration, PNAS, 102:15629–15634, 2005.

55. Shi L., Sohaskey C.D., Pfeiffer C., Datta P., Parks M., McFadden J., North R.J. and Gennaro M.L., Carbon flux rerouting during *Mycobacterium tuberculosis* growth arrest, Mol Microbiol. , 78:1199-215, 2010.

56. Forrellad M.A.,Klepp L.I., Gioffre A., Garcia J.S.y, Morbidoni H.R., Santangelo M., Cataldi A.A. and Bigi F., Virulence factors of the *Mycobacterium tuberculosis* complex, Virulence, 4: 3-66, 2013.

57. Rajni, Rao N. and Meena L.S., Biosynthesis and Virulent Behaviour of Lipids Produced byMycobacterium tuberculosis: LAM and Cord Factor: An Overview, Biotechnology Research International, 2011:1-7, 2011.

58. Akif M., Khare G., Tyagi A.K., Mande S.C. and Sardesai A.A., Functional Studies of Multiple Thioredoxins from *Mycobacterium tuberculosis,* journal of bacteriology, 190:7087-7095, 2008.

59. Brandes N., Reichmann D., Tienson H., Leichert L.I. and Jacob U., Using Quantitative Redox Proteomics to Dissect the Yeast Redoxome, J. Biol. Chem., 286:41893-903, 2011.

60. Cotgreave, I. A., Gerdes, R., Schuppe-Koistinen, I., and Lind, C. (2002) Methods Enzymol348, 175-182.

61. Elsen S., Efthymiou G., Peteinatos P., Diallinas G., Kyritsis P. and Moulis J., A bacteria-specific 2[4Fe-4S] ferredoxin is essential in *Pseudomonas aeruginosa*, BMC Microbiology, 10:271, 2010.

62. Chauhan R. and Mande S.C., Characterization of the *Mycobacterium tuberculosis* H37Rv alkyl hydroperoxidase AhpC points to the importance of ionic interactions in oligomerization and activity, Biochem. J., *354*:209-215, 2001.