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Strategies Employed by *Mycobacterium Tuberculosis* $H_{37}Rv$ for Survival at Low pH

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Abbreviations

Tuberculosis (TB); *Mycobacterium tuberculosis* $H_{37}Rv$ (*M. tuberculosis*); Lysosomal-associated membrane protein 1 (LAMP-I); N, N′-dicyclohexyl Carbodiimide (DCCD); *Mycobacterium marinum* (*M. marinum*); Tryptophan-Aspartate Coat Protein (TACO)

Abstract

Tuberculosis (TB) is a deadly infectious disease caused by *Mycobacterium tuberculosis* $H_{37}Rv$ (*M. tuberculosis*) which enters the respiratory tract of a healthy person through respiration. It reaches the lung epithelial cells and ultimately encounters the lung macrophages. Macrophages are very effective in phagocytosis and clearing out bacteria but *M. tuberculosis* has the potential to survive even in the extremely punitive environment. The pH of macrophage in which *M. tuberculosis* resides is very low. The ability of *M. tuberculosis* to survive in such acidic atmosphere makes it distinct from other pathogens. This review focuses on the strategies employed by *M. tuberculosis* for survival at low pH such as: (a) modification of serine residue; (b) secretion of ammonia; (c) presence of intact cell wall (d) extrusion of protons (e) remodelling of gene expression; and (f) alteration of phagosome.

Keywords: Lysosome; Macrophage; pH Regulation; Phagosome; Proton Extrusion

Introduction:

Tuberculosis (TB) is the leading cause of death in the world due to single infectious bacterium. Millions of people fall sick with TB each year. World Health Organisation (WHO) estimated that 10 million people across the globe developed TB in the year 2017 [1]. TB is caused by *Mycobacterium tuberculosis* $H_{37}Rv$ (*M. tuberculosis*) which is released into the atmosphere in the form of air droplets, either by sneezing or coughing of infected person. They enter the host through respiration and ultimately reach the lungs where they cause primary infection. Thereafter, they move to the lymphoid organs and other parts of the body where they cause secondary infection [2]. *M. tuberculosis* has the special ability to survive inside the macrophage and use it for replication. This ability of *M. tuberculosis* makes it a highly successful pathogen [3]. The pH of macrophage in which *M. tuberculosis* resides ranges from 6.2 to 4.5. Therefore, *M. tuberculosis* must maintain their acid-base homeostasis to survive [4]. The strategies adapted by *M. tuberculosis* to survive in such low pH include inhibition of acidification of the phagosome and inhibition of phagosome-lysosome fusion [5]. Moreover, *M. tuberculosis* has the potential of remodelling its gene expression to exhibit growth inside the phagosome [6]. *M. tuberculosis* can maintain its intra-bacterial pH and thus, resist the phagolysosomal concentrations of acid. Therefore, disruption of the systems which are responsible for *M. tuberculosis* acid resistance and intra-bacterial pH resistance can prove to be an attractive target for chemotherapy for the treatment of tuberculosis. Many genes have been identified that are induced by acidic pH in the phagosome. These induced genes can be excellent candidates for genes that are required for growth inside the macrophage [7]. Homeostasis of intracellular pH is an important feature of *M.tuberculosis* to survive inside the macrophages. Therefore, in this study, we have discussed the mechanisms adapted by *M. tuberculosis* to survive within the acidic environment inside the macrophage.

Modification of Serine Residue:

Rv3671c gene in *M. tuberculosis* is essential for tolerance and survival in the acidic conditions which are encountered inside the macrophage. Rv3671c encodes a membrane associated serine hydrolase with conserved aspartate, histidine and serine active site residues and four transmembrane domains which are required for survival inside the host [8, 9]. When the Rv3671c membrane protein is mutated, a loss of cytoplasmic pH homeostasis occurs which result in strong reduction of virulence of *M. tuberculosis* [10]. *M. tuberculosis has* the ability to maintain its intra-bacterial pH near neutral in the low pH environment of phagosomes within activated macrophages. It has been reported by genetic screening that *M. tuberculosis* loses this ability when the mycobacterial acid resistance serine protease (marP) gene is disrupted [11]. Pathogenic mycobacteria resist delivery to lysosomes after uptake into macrophages which allows them

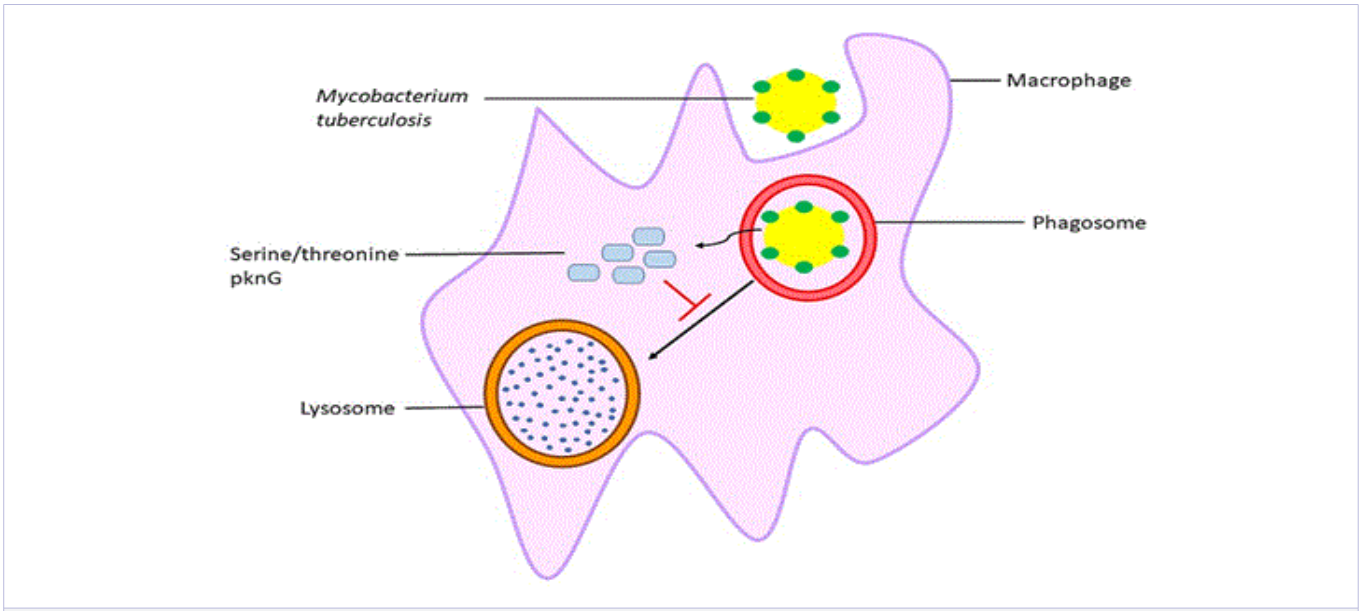

Figure 1: Modification of serine residue: *M. tuberculosis* when present inside the phagosome release serine/threonine protein kinase G which inhibits the fusion of phagosome with lysosomes

to survive. It has been found that the eukaryotic-like serine/threonine protein kinase G is secreted by mycobacteria inside phagosomes. This inhibits phagosome-lysosome fusion and mediates the intracellular survival of mycobacteria. Inactivation of protein kinase G by gene disruption or chemical inhibition results in mycobacterial cell death [12].

Secretion of Ammonia:

M. tuberculosis can produce plenty of ammonia, which performs inhibitory effect on phagolysosomal fusion [3]. Ammonia secretion is a mechanism by which *M. tuberculosis* neutralizes the surrounding acidic environments. *OmpATb* operon is necessary for rapid ammonia secretion and adaptation of *M. tuberculosis* to acidic environments in vitro. Chemical analysis of low pH culture shows that the proteins encoded by the *ompATb* operon are involved in generating a rapid ammonia burst. This neutralises the acidic pH and leads to exponential growth of *M. tuberculosis* [13]. Another mechanism by which ammonia neutralises the pH involves the entry of asparagine to *M. tuberculosis* phagosome. Asparagine is captured by *M. tuberculosis* through AnsP2 receptor and then hydrolysed by cytosolic AnsA enzyme. This results in the formation of glutamine and glutamate, along with release of ammonia. AnsA can also hydrolyse asparagine in the lumen of phagosome, resulting in the production of aspartate and ammonia. Ammonia reacts with the protons that are transported by V-ATPase and forms ammonium ions. This leads to the neutralisation of the phagosomal pH [14]. (Figure2)

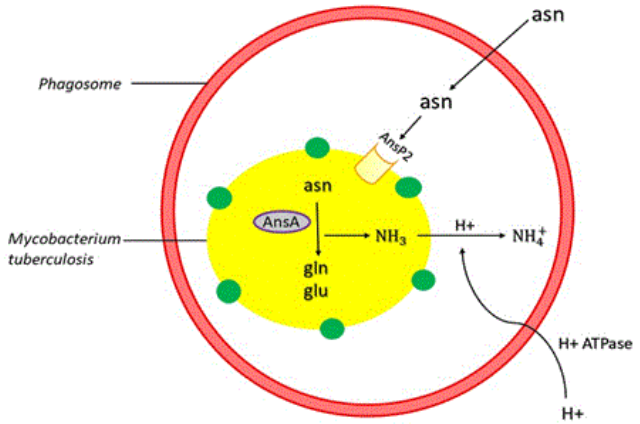

Figure 2: Secretion of ammonia: Asparagine enters the phagosome where it is captured by the AnsP2 receptor of *M. tuberculosis.* Asparagine is converted into glutamine and glutamic acid with the help of enzyme AnsA. NH_3 is released as a by-product which is converted into NH_4^+ due to the presence of H+ ions inside the phagosome.

Presence of intact cell wall:

M. tuberculosis has a lipid rich cell envelope that acts as an effective barrier against the entry of protons [15]. The cell wall consists of a core comprised of peptidoglycan which is attached covalently to a linear galactofuran which in turn is attached to several strands of a highly branched arabinofuran which is further attached to mycolic acids. The mycolic acids are aligned perpendicular to the membrane's plane and form a special lipid barrier [16]. In the early 1900s, Metchnikoff hypothesized that the waxy *M. tuberculosis* cell wall is an important protector against acid stress present in phagocytes. Recent work has shown the presence of

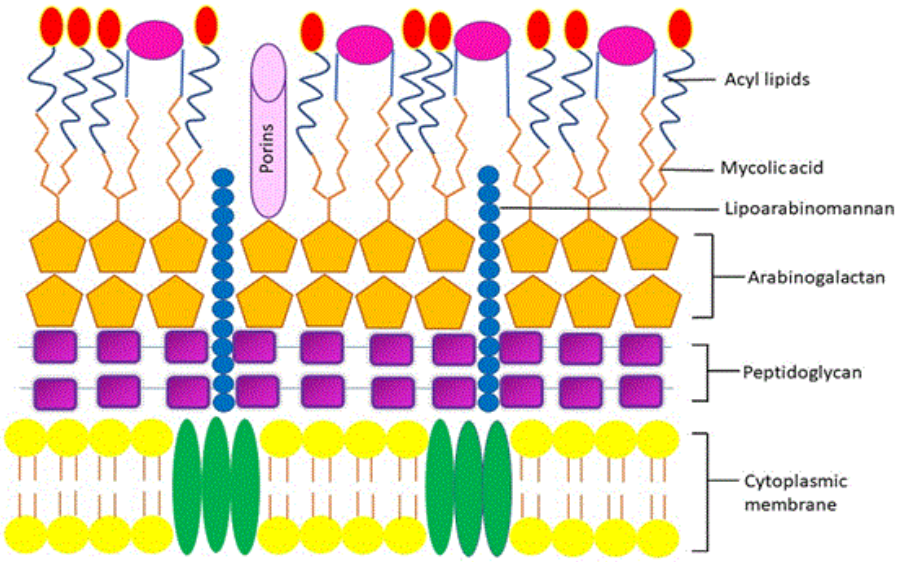

Figure 3: ***Cell wall of M. tuberculosis:*** The cell wall of *M. tuberculosis* has a rich network of lipid content and thus, protects it from the low pH of phagosome and lysosome.

an additional outer lipid bilayer that surrounds the mycobacteria. This cell envelope acts as a forbidding permeability barrier for protons. Studies that examine the physiology of mycobacteria at low pH show that the cell wall certainly plays an important role in acid resistance [17]. A lot of data is available in this regard which show experimental proof of the permeability defect on cell wall using hypersensitivity to lipophilic antibiotics as a measure. Rv3671c and lysX mutants were revealed to be hypersensitive to lipophilic antibiotics. Rv2136c, Rv2224c, and ponA2 mutants were also proved to be hypersensitive to the lipophilic antibiotics erythromycin and rifampicin. Along with this, Rv2136c and Rv2224c mutants were also revealed to be hypersensitive to the cell wall-perturbing detergent SDS [18].

Extrusion of proteins

When engulfment of mycobacteria occurs, vacuoles acidify rapidly which indicates that proton-ATPase is delivered early to the endosome or internalized with the plasmalemma. Observations on phagosomes indicate that shortly after internalization proton ATPase is accumulated. Mycobacterium selectively inhibits the fusion of its vacuole with proton-ATPase-positive vesicles, while actively fusing with lysosome-associated membrane proteins 1 (LAMP-l) carrying vesicles. This suggests that either a selective inhibition of fusion with proton-ATPase-containing vesicles takes place or rapid removal of this complex from Mycobacterium phagosomes occurs [19]. Probably, the exclusion of vacuolar proton-ATPase causes the lack of acidification. At external pH 5, the change in pH of *M. smegmatis* is dissolute by using protonophores like carbonyl cyanide m-chlorophenylhydrazone, ionophores like monensin and nigericin and N, N'-dicyclohexyl carbodiimide (DCCD) which is an inhibitor of the proton-translocating F1F0-ATPase. This demonstrates that the permeability of cytoplasmic membrane to protons and extrusion of protons by the F1F0-ATPase plays an essential role in maintaining internal pH near neutral [20].

Remodelling of Gene Expression:

After phagocytosis of *M. tuberculosis* by macrophages, *M. tuberculosis* senses the intracellular environment and remodels its gene expression to grow inside the phagosome. An acid and phagosome regulated locus (aprABC) has been identified. It is very unique to *M. tuberculosis* complex and its gene expression is induced during growth in acidic environment. Expression of aprABC is dependent on the regulator phoPR. This creates a link between phoPR signalling and pH sensing (Fig 5) [7]. Several genes have been identified which are favourably expressed when *Mycobacterium marinum (M. marinum)* resides in the host granulomas or macrophages.

Two of the genes of *M. marinum* were found to be *M. tuberculosis* Pro-Glu/Pro-Glu Polymorphic GC-rich Sequence (PE/PE_PGRS) gene homologs. The mutation of these two genes of *M. marinum* wild type strains of PE_PGRS family produced *M. marinum* strains that were incapable of replication in macrophages. The strains exhibited decreased persistence in granulomas, thereby suggesting a direct role for PE_PGRS proteins in mycobacterial virulence [3].

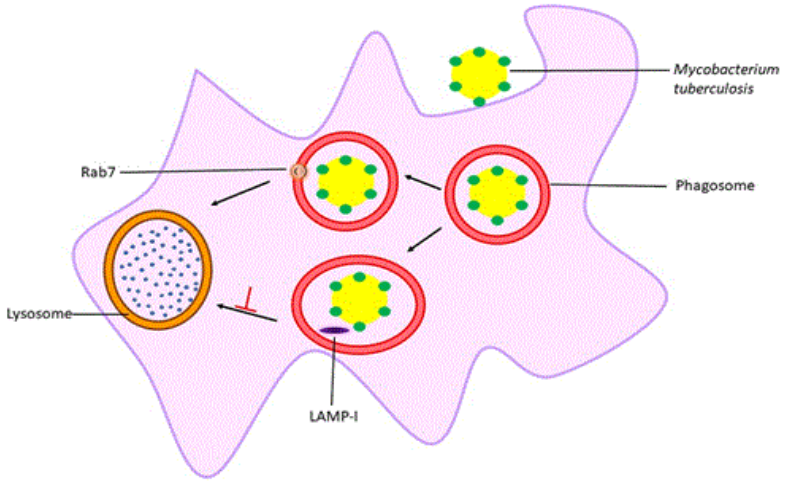

Figure 4: Extrusion of protons: When the phagosome containing *M. tuberculosis* shows the presence of LAMP-I, inhibition of fusion of phagosome and lysosome is observed whereas when phagosome shows the presence of Rab7, active fusion of phagosome and lysosome is observed.

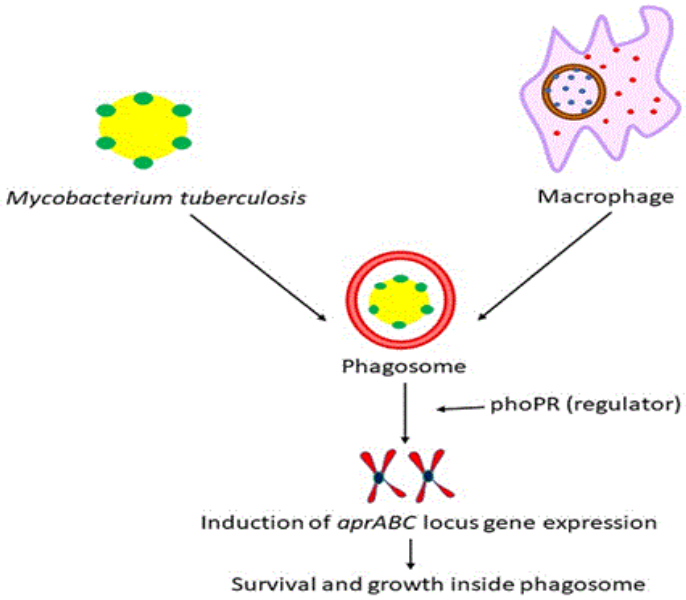

Figure 5: Remodelling of gene expression: Phagosome containing *M. tuberculosis* shows the induction of aprABC locus gene expression in the presence of phoPR regulator. This helps in the survival and growth of *M. tuberculosis* inside the phagosome.

Alteration of Phagosome:

Along with modifications in its own structural and functional features at low pH, *M. tuberculosis* also modifies the phagosome of the host to enhance its survival. These alterations in the phagosomes do not let them fuse with lysosomes and let them only be mildly acidified. The perseverance of Rab5 on the *M. tuberculosis* phagosomes enables the phagosome at an early endosomal stage to stop its own maturation [21]. Another strategy used by *M. tuberculosis* involves the recruitment of a tryptophan aspartate repeat host protein to the phagosomes containing mycobacteria. The active retention of Tryptophan-Aspartate containing CO at protein (TACO) on phagosomes by living mycobacteria thus is an important mechanism that prevents the delivery to phagosomes to lysosomes, allowing mycobacteria to survive [22].

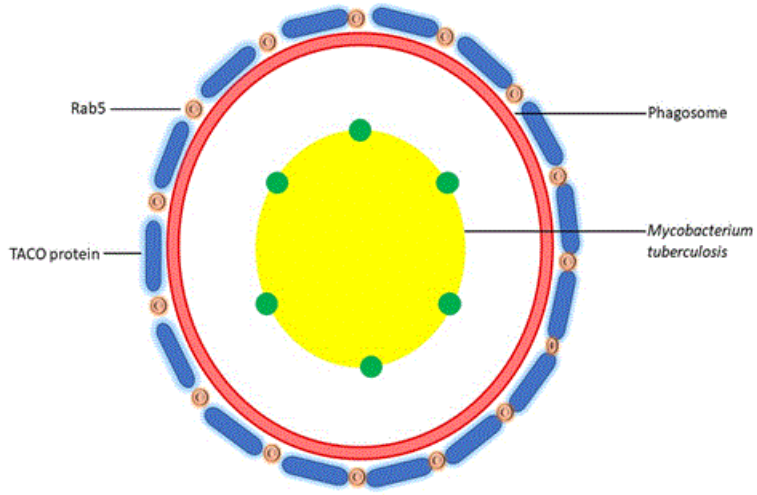

Figure 6: Alteration of phagosome: The retention of Rab5 and TACO protein on the phagosome containing *M. tuberculosis* inhibit its fusion with the lysosomes

Discussion

TB is an airborne disease caused by infectious *M. tuberculosis* being inhaled as air droplets which have been exhaled by infected person into the environment. As the bacteria approach the lungs, they are phagocytosed by alveolar macrophages. *M. tuberculosis* has the ability to utilise the host's macrophages for its replication. Despite encountering the low pH of macrophages, *M. tuberculosis* still survives. *M. tuberculosis'* ability to persist in such acidic environment is creditable to its highly efficient intracellular pH regulation system. There are various strategies used for the homeostasis of intracellular pH such as serine residue modification, ammonia secretion, intact cell wall, proton extrusion, gene expression remodelling and alteration of phagosome. In this review, we have discussed the above-mentioned strategies in brief so that these can be targeted to disrupt its survival properties.

Acknowledgement

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Conflict of Interest

No conflict of interest.

References

1. Laura Anderson, Annabel Baddeley, Hannah Monica Dias, Katherine Floyd, Inés Garcia Baena, Nebiat Gebreselassie, etal., Global Tuberculosis Report 2018. World Health Organisation.2018; 1-277.
2. Meena S, Meena LS. To Understand the Role of Heme Molecules in the Survival and Pathogenesis of *Mycobacterium tuberculosis*. Journal of Tuberculosis and Therapeutics. 2017; 2(1):1-3.
3. Meena LS, Rajni. Survival mechanisms of pathogenic *Mycobacterium tuberculosis* $H_{37}Rv$. The FEBS Journal.2010; 277(11): 1-12. doi:10.1111/j.1742-4658.2010.07666.x
4. Vandal OH, Nathan CF & Ehrt S .Acid resistance in *Mycobacterium tuberculosis*. Journal of Bacteriology.2009; 191(15): 4714-4721. doi:10.1128/JB.00932-08
5. Gomes MS, Paul S, Moreira AL, Appelberg RUI, Rabinovitch M & Kaplan G. Survival of *Mycobacterium avium* and *Mycobacterium tuberculosis* in Acidified Vacuoles of Murine Macrophages. Molecular and Cellular Pathogenesis.1999; 67(7): 3199-3206.
6. Khan MZ, Bhaskar A, Upadhyay S, Kumari P, Rajmani RS, Jain P et-al. Protein kinase G confers survival advantage to *Mycobacterium tuberculosis* during latency-like conditions. Journal of Biological Chemistry. 2017; 292(39): 16093-16108. doi:10.1074/jbc.M117.797563
7. Abramovitch RB, Rohde KH, Hsu FF, & Russell DG. apr ABC: a *Mycobacterium tuberculosis* complex-specific locus that modulates pH-driven adaptation to the macrophage phagosome. MolecularMicrobiology.2011; 80(3):678-694. doi:10.1111/j.1365-2958.2011.07601.x
8. Nathan C, Ehrt S, Vandal O, & Darby C U.S. Patent No. 8,951,992. Washington, DC: U.S. Patent and Trademark Office.2015;
9. Vandal OH, Pierini LM, Schnappinger D, Nathan CF, & Ehrt S. A membrane protein preserves intrabacterial pH in intraphagosomal *Mycobacterium tuberculosis*. Nature Medicine.2008;14(8): 849-854. doi:10.1038/nm.1795
10. Baker JJ, Johnson BK & Abramovitch RB .Slow growth of *Mycobacterium tuberculosis* at acidic pH is regulated by phoPR and host-associated carbon sources. *Molecular Microbiology*.2014; 94(1): 56-69. doi:10.1111/mmi.12688
11. Zhao N, Darby CM, Small J, Bachovchin DA, Jiang X, Burns-Huang KE

et-al. Target-based screen against a periplasmic serine protease that regulates intrabacterial pH homeostasis in *Mycobacterium tuberculosis*. ACS Chemical Biology.2014; 10(2): 364-371. doi:10.1021/cb500746z

12. Walburger A, Koul A, Ferrari G, Nguyen L, Prescianotto-Baschong C, Huygen K et-al. Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. Science.2004; 304(5678): 1800-1804.doi: 10.1126/science.1099384
13. Song H, Huff J, Janik K, Walter K, Keller C, Ehlers S & Niederweis M . Expression of the ompATb operon accelerates ammonia secretion and adaptation of *Mycobacterium tuberculosis* to acidic environments. Mol Microbiol.2014; 80(4):900-918. doi:10.1111/j.1365-2958.2011.07619.x
14. Gouzy A, Larrouy-Maumus G, Bottai D, Levillain F, Dumas A, Wallach JB ,etal. *Mycobacterium tuberculosis* exploits asparagine to assimilate nitrogen and resist acid stress during infection. PLoS Pathogens.2014;10(2): doi: 10.1371/journal.ppat.1003928
15. Élie Metchnikoff. Immunity in infective diseases. University Press.1905; 1-617.
16. Brennan PJ. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. Tuberculosis.2003; 83(1-3): 91-97. doi:10.1016/s1472-9792(02)00089-6
17. Hoffmann C, Leis A, Niederweis M, Plitzko JM and Engelhardt H. Disclosure of the mycobacterial outer membrane: cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. Biology, Medicine.2008; 105(10):3963-3967. doi:10.1073/pnas.0709530105
18. Vandal OH, Roberts JA, Odaira T, Schnappinger D, Nathan CF and Ehrt S .Acid-susceptible mutants of *Mycobacterium tuberculosis* share hyper susceptibility to cell wall and oxidative stress and to the host environment. J Bacteriol.2009; 191(2): 625-631. doi: 10.1128/JB.00932-08
19. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK et-al. Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. Science.1994; 263(5147):678-681. doi:10.1126/science.8303277
20. Rao M, Streur TL, Aldwell FE & Cook GM . Intracellular pH regulation by *Mycobacterium smegmatis* and *Mycobacterium bovis* BCG. Microbiology.2001; 147(4): 1017-1024. doi:10.1099/00221287-147-4-1017
21. Clemens DL, Lee BY & Horwitz MA. Deviant Expression of Rab5 on Phagosomes Containing the Intracellular Pathogens *Mycobacterium tuberculosis* andLegionella pneumophila is Associated with Altered Phagosomal Fate. Infect Immun.2000; 68(5): 2671-2684. doi:10.1128/iai.68.5.2671-2684.2000
22. Ferrari G, Langen H, Naito M, Pieters J.A coat protein on phagosomes involved in the intracellular survival of mycobacteria. Cell.1999; 97(4): 435-447.doi: 10.1016/s0092-8674(00)80754-0