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Suppression of Eis and expression of Wag31 and GroES in *Mycobacterium tuberculosis* cytosol under anaerobic culture conditions†

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A major impediment in chemotherapy of Tuberculosis (TB) is the persistence of *M. tuberculosis* in a latent or dormant state, possibly perpetuated by paucity of oxygen within the lung granuloma. Proteome analysis of the anaerobically persisting microbe could therefore provide novel targets for drugs against latent TB infection (LTBI). An Indian clinical isolate of *M. tuberculosis* was cultured under aerobic and anaerobic conditions following Wayne's hypoxia model and its cytosolic proteins were resolved by two-dimensional gel electrophoresis (2DE). Peptide mass fingerprinting of 32 differentially expressed spots using MALDI TOF-TOF MS-MS resulted in identification of 23 proteins. Under the anaerobic culture conditions, expression of 12 of these proteins was highly suppressed (>2 fold reduction in spot volumes), with 4 of them (GrpE, CanB, MoxR1 and Eis) appearing as completely suppressed since corresponding spots were not detectable in the anaerobic sample. On the other hand, 4 proteins were highly expressed, with two of them (Wag31 and GroES) being uniquely expressed under anaerobic conditions. Suppression of Eis could make the anaerobically persisting bacilli susceptible to the aminoglycoside antibiotics which are known to be acetylated and inactivated by Eis. Although all 4 over-expressed proteins can be considered as putative drug targets for LTBI, Wag31 appears particularly interesting in view of its role in the cell wall biogenesis.

Keywords: Anaerobic persistence, Eis, GroES, *Mycobacterium tuberculosis*, LTBI, TB, Wag31

Over a third of world's population harbors 'latent' tuberculosis infection (LTBI) and, from this vast pool of potential TB cases, 5-10% develop active disease during their lifetime[1]. Primary infection with *Mycobacterium tuberculosis* typically leads to replication of bacilli inside alveolar macrophages until an effective immune response restricts them to the granulomas. Though the environment within a granuloma is considered as hostile, the bacilli can survive in it for long durations[2]. These latent (or dormant) bacilli are unresponsive to currently available chemotherapy and remain a potential source of activation of the disease[1]. Elucidation of *M. tuberculosis* genes and proteins which are specifically modulated during latency is therefore considered essential for identification of potential targets for new drugs against LTBI[3].

Relative avascularity and poor permeability of the granuloma strongly suggest that scarcity of oxygen is a major factor that drives *M. tuberculosis* bacilli into latency[3,4]. Wayne[5] demonstrated that bacilli that settle through a 'self-generated' oxygen depletion gradient undergo an orderly metabolic shift-down. Accumulating at the bottom of culture tube, they enter a homogeneous physiologic state of dormancy and exhibit a 'synchronous' replication cycle upon re-exposure to air[6]. In this state of 'non-replicating persistence (NRP)' most RNA and protein synthesis stops, though there is an enhanced production of isocitrate lyase (Icl), glycine dehydrogenase, Acr and few other proteins[7,8]. The bacilli become resistant to many antibiotics, including isonizaid and rifampicin, but show susceptibility to metronidazole[9]. A subsequent study using knockout of Icl has confirmed the importance of glyoxylate pathway in persistence of *M. tuberculosis*[10].

In a proteomic study based on Wayne's model of LTBI, Cho *et al.*[11] subjected *M. tuberculosis* cell lysates to Isotope Coded Affinity Tagging (ICAT) in order to identify proteins which were expressed differentially during NRP stages. Nonetheless, ICAT technology has some inherent limitations. It requires presence of cysteine in the protein whereas over 18% of *M. tuberculosis* proteins lack cysteine residues[11]. Another evaluation has shown that the ICAT method under-represents small proteins and is biased towards

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