Role of Internal Carbon Mobilization for an Optimal Re-Growth of Dormant Mycobacterium tuberculosis

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Abstract

*Mycobacterium tuberculosis* (Mtb) is the bacteria that results in the pathogenic infection of Tuberculosis, a serious pulmonary infection that is fatal if left untreated. In the presence of antibiotics, Mtb enters a persister state, in which the bacteria is metabolically dormant and the infection subsides. However, upon the elimination of antibiotics, Mtb exits the persister state and returns back to a virulent state. We hypothesize that this reactivation process of Mtb includes the internal consumption of upstream glycolytic metabolites as a source of downstream glycolytic intermediates as upstream ones are highly accumulated with reciprocal depletion of downstream ones during the dormant stage, and during re-activation these metabolites. This study examined the role of accumulated glycolytic intermediates as a source of internal carbon mobilization for an optimal re-growth of dormant Mtb. For this, we used hypoxia as this condition engendered Mtb into a dormant state, and metabolomic remodeling during re-growth was analyzed through LCMS mediated metabolomics. The outcome of this study will uncover new therapeutic targets to clear Mtb infection.

1. **Mtb Culture**
   - a. Strains of Mtb → RV (Wildtype), RV ATC, PYK (pyk knockdown), PYK ATC, PYK PYK+ATC.
   - b. Utilized m7H9 or m7H10 growth media
   - c. Created mutant strains via Crispr–dCas9
   - d. Filter culture system to give hypoxia for re-activation

2. **Checking Reactivation Growth**
   - a. Replicating culture:
      - i. Bacteria set to optical density of 0.05 (day 0)
      - ii. Optical density checked every 3-4 days to record replication growth
   - b. Reactivation culture:
      - i. Re-activation culture created through disturbing Mtb filters upon 3 days of hypoxic conditions
      - ii. Optical density checked every 3-4 days to record reactivation growth

3. **LC-MS Metabolomics**
   - a. Samples for metabolomics transferred to C13 isotopologues for detection of internal vs External carbon
   - b. Liquid Chromatography Mass Spectrometry used to detect metabolites present in replication and re-grown samples
   - c. Principal component analysis and Heat Map analysis performed via MetaBioanalyst V.5.0

Fig. 1.1. Cycle of replication, dormancy, and re-activation in vivo of *Mycobacterium tuberculosis*

Fig. 2.1. - Replication growth in comparison to the reactivation growth of mtb wild type strain (RV). As highlighted in the yellow circle, the replication growth has a substantially higher OD from days 3 to 6, as the re-activation growth is experiencing a lag phase.

Fig. 2.2. - The metabolomic data of RV reactivation. The kinetic charts depict elevated levels of G6P, PEP, S7P, and TrioseP at 0 hour time point, and upon exit from hypoxia depict significant depletion. This is resulting from Mtb accumulating these upstream glycolytic metabolites during persister state, and upon reactivation utilizing these metabolites for biosynthesis of PEP, thus triggering the Citric Acid cycle. The make up of the carbon source within 0 hour metabolite amongst G6P, PEP, S7P, and TrioseP is majority unlabeled, this indicates that it is an internal carbon source accumulation, compared to exit from hypoxia, in which majority of the metabolite is labeled indicating external C13 glucose as the carbon source.

Fig 3.1 – Left – Central carbon metabolism (e.g., glycolysis and pentose phosphate pathway) and cell wall glycolipid precursor metabolism (e.g., trehalose or alpha glucan) belong to high ranked pathways that were different between the two conditions. Right – PCA analysis showed the Mtb metabolic networks in a replication and a re-growth condition were clearly different.

Fig 4.2. PYK blocks the conversion of PEP into pyruvate, shown by the depletion of pyruvate and build up of PEP in PYK reactivation. Mtb PYK re-directs the upstream glycolytic metabolites to the PPP pathway, this is shown as PEP and S7P key metabolites in the PPP pathway - exhibit elevated levels within the PYK reactivation. Additionally, metabolites within the Citric Acid cycle, specifically succinate and alpha ketoglutarate depleted levels within PYK reactivation. This explains the significant latency of the PYK reactivation growth in comparison to the RV reactivation growth.

**Method**

1. Replicating culture:
   - a. Replicating culture: Bacteria set to optical density of 0.05 (day 0)
   - b. Replicating culture: Optical density checked every 3-4 days to record replication growth

2. Reactivation culture:
   - a. Reactivation culture:
      - i. Reactivation culture created through disturbing Mtb filters upon 3 days of hypoxic conditions
      - ii. Reactivation culture:
         - Optical density checked every 3-4 days to record reactivation growth

3. LC-MS Metabolomics:
   - a. Samples for metabolomics transferred to C13 isotopologues for detection of internal vs External carbon
   - b. Liquid Chromatography Mass Spectrometry used to detect metabolites present in replication and re-grown samples
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**References**