Probing mycobacterial metabolism in tuberculosis and leprosy to identify vulnerable metabolic nodes for drug development

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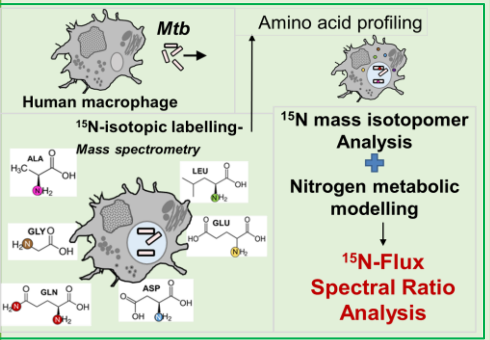
Background: Metabolism of pathogens in infectious diseases is important for their survival, virulence and pathogenesis. Mycobacterial pathogens successfully scavenge multiple host nutrient sources in their intracellular niche. It is therefore important to identify the intracellular nutrient sources and their metabolic fates in these pathogens. We quantified *in vivo* fluxes of the pathogens and probed host-bacterial metabolic cross talks in tuberculosis (TB) and leprosy using systems-based approaches of isotopic labelling, metabolic modelling and metabolic flux analysis.

Tuberculosis (TB): We developed ^{15}N-flux spectral ratio analysis (^{15}N-FSRA) to measure nitrogen metabolism of *Mycobacterium tuberculosis* (Mtb) in human macrophages [1].

Methodology:



Results:

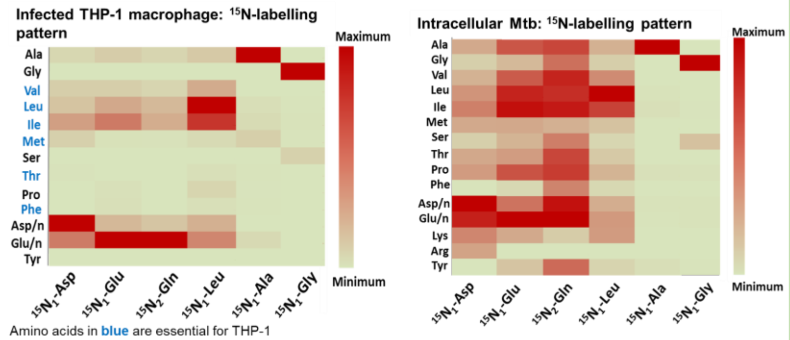


Fig. Assimilation Pattern of Different Nitrogen Sources. Heatmaps are shown for amino acids derived from Mtb-infected macrophages and intracellular Mtb. Assimilation patterns are shown for six tracers: $^{15}N_1$-Asp, $^{15}N_1$-Glu, $^{15}N_2$-Gln, $^{15}N_1$-Leu, $^{15}N_1$-Ala, and $^{15}N_1$-Gly. Values are mean ± SEM from 3–4 independent infections and labelling experiments [1]. alanine (Ala); glycine (Gly); valine (Val); leucine (Leu); isoleucine (Ile); methionine (Met); serine (Ser); threonine (Thr); proline (Pro); phenylalanine (Phe); aspartate/asparagine (Asp/n); glutamate/glutamine (Glu/n); lysine (Lys); arginine (Arg); tyrosine (Tyr)

Results:

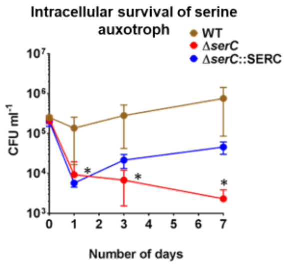
Table 1. Flux Spectral Ranges Determined with ^{15}N FSRA

Category	Nitrogen Source	Flux Spectral Range: Minimum Ratio	Maximum Ratio	Median
↓	Asn	0.06	0.19	0.13
↓	Pro	0.31	0.38	0.34
↓	Thr	0.37	0.49	0.43
↓	Ile	0.52	0.64	0.55
↓	Tyr	0.57	0.63	0.60
↓	Leu	0.58	0.96	0.61
↓	Met	0.64	0.77	0.68
↓	Phe	0.63	0.75	0.69
↓	Lys	0.78	0.82	0.81
→	Ala	0.98	1	1
0/○	Glu	0	1.63	0.02
0/○	Ser	0	3.59	1.31
○	Gly	0.51	3.17	0.67
○	Asp	0.61	12.46	2.11
↑	Gln	1.50	11.85	2.47
↑	Val	1.82	13.45	2.88

↓ spectral ranges in the interval [0,1), the biomass nitrogen need has to be fulfilled by *de novo* synthesis of the amino acid
→ spectral range is essentially 1, nitrogen biomass requirement is balanced with uptake
0 the amino acid is not taken up
○ spectral range is inconclusive
↑ spectral ranges are larger then 1, indicating that this amino acid is a nitrogen donor

Asparagine (Asn); glutamine (Gln)

Results:



Growth of WT, ΔserC, and ΔserC::SERC in THP-1 macrophages. CFUs (colony forming units) of the three strains were recorded for 0 (before infection), 1, 3, and 7 days post-infection. Data are the average of six independent infection experiments, each with three technical replicates

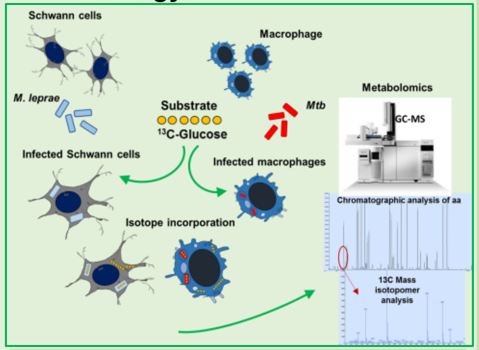
Conclusions:

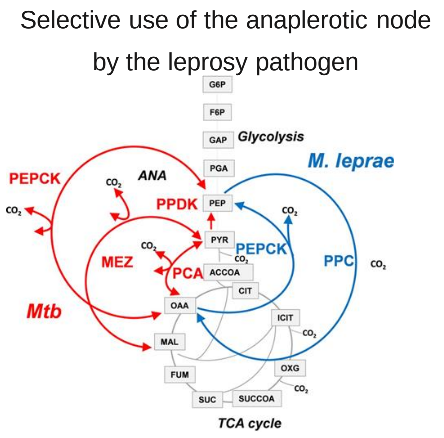
- Mtb utilizes multiple amino acids as nitrogen sources in human macrophages
- ^{15}N-FSRA tool identified the intracellular nitrogen sources available to Mtb in macrophages
- Glutamine is the predominant nitrogen donor for Mtb
- Serine biosynthesis is essential for the survival of intracellular Mtb
- SerC is a drug target

[1] Borah et al., 2019, Cell Reports, Volume 29, Issue 11, 3580-3591.

Leprosy: We applied metabolomics and ^{13}C isotopomer analysis to measure carbon metabolism of the pathogen *Mycobacterium leprae* (Mlep), in its primary host cell, the Schwann cell and compared it with its related mycobacterial TB pathogen growing in a macrophage [2].

Methodology:

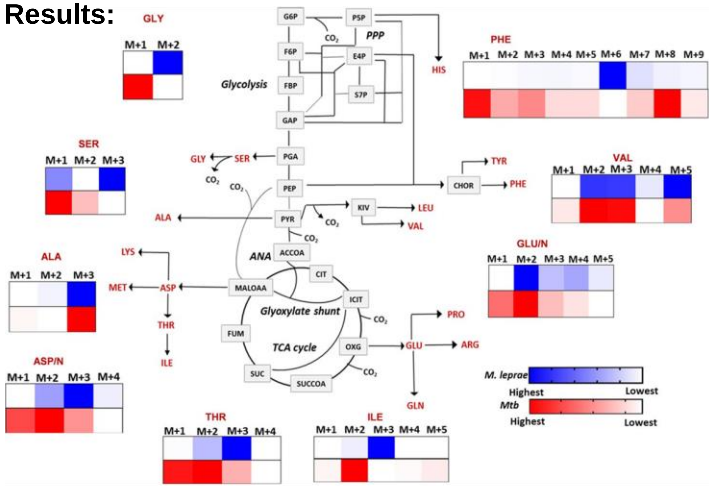




[2] Borah et al., 2019, mBio: 10:e02351-19

Differences between Mlep and Mtb intracellular glucose metabolism

Results:



^{13}C isotopomer profiles of amino acids in intracellular Mlep vs. Mtb. Amino acid profiles are plotted on a metabolic map with M1, M2, M3, M4, M5, M6, M7, M8, and M9 mass isotopomer families. Proportional increases or decreases in the ^{13}C abundance of mass isotopomers of a metabolite are indicated by a single gradient. Measurements are averages ± SD from three independent infection experiments.

Conclusions:

- Mtb imports most of its amino acids directly from the host macrophage, BUT Mlep utilizes host glucose pools as the carbon source to biosynthesize its amino acids.
- The anaplerotic enzyme phosphoenolpyruvate carboxylase is required for this intracellular diet of Mlep and is a potential anti-leprosy drug target.