Nitrogen metabolism in *Mycobacterium tuberculosis*: a systems-based approach

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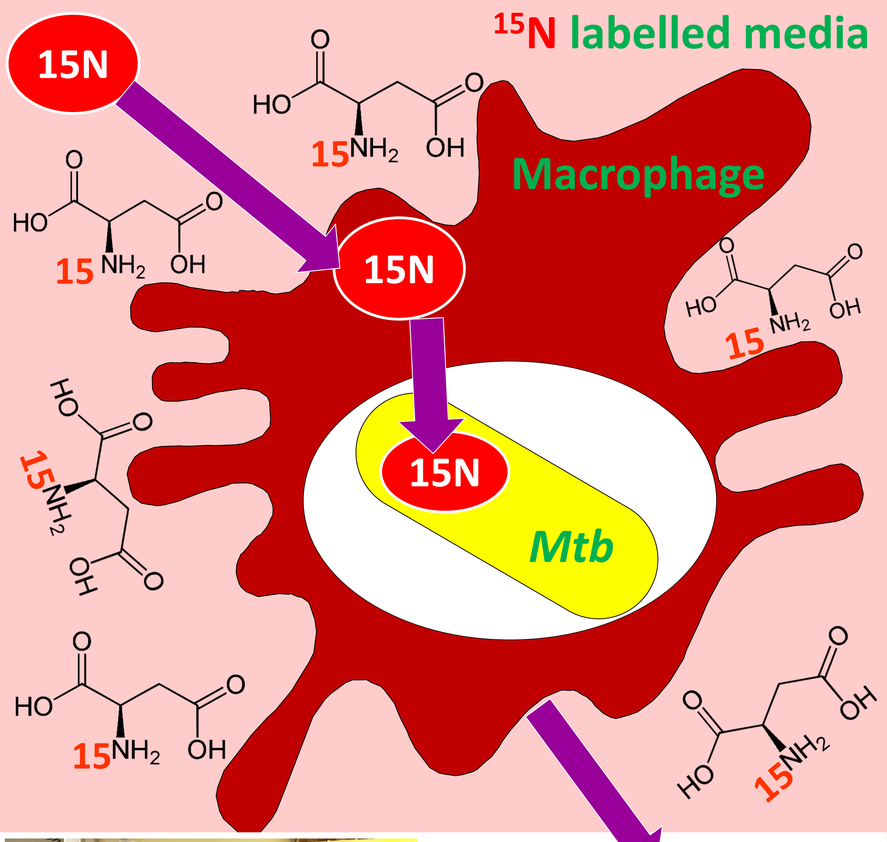
INTRODUCTION

- Several studies have already shown *Mycobacterium tuberculosis (Mtb)* obtains its carbon from host-derived nutrients and exploits specific metabolic pathways during infection
- In comparison to carbon, the processes *Mtb* uses to acquire and assimilate nitrogen (N) from the host is poorly studied despite N also being an essential component of biomolecules
- Our previous studies suggested the hypothesis that *Mtb* obtains N from a diverse range of intracellular nutrients including amino acids
- Here, we use a novel system's based three-pronged approach to define pathways for uptake and assimilation of N. This consists of transposon mutagenesis, ^{15}N isotopologue profiling of intracellular *Mtb*, and performing flux analysis using $^{13}C/^{15}N$ isotopologue profiling in continuous culture

THE SYSTEMS BIOLOGY STRATEGY

^{15}N isotopic profiling *Ex vivo Mtb*



GC-MS

Isotopic profiling of amino acids

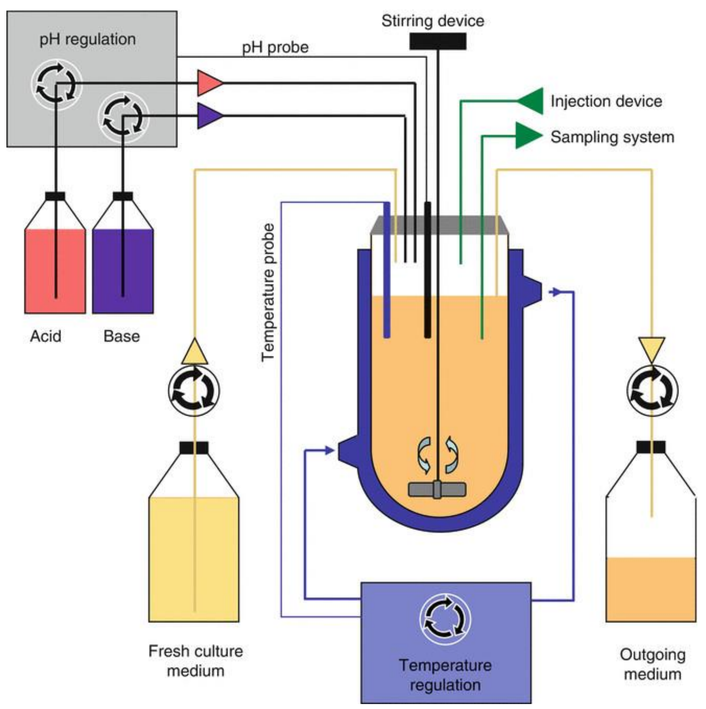
Differential Centrifugation

Extracellular *Mtb*

Intracellular *Mtb*

Macrophage

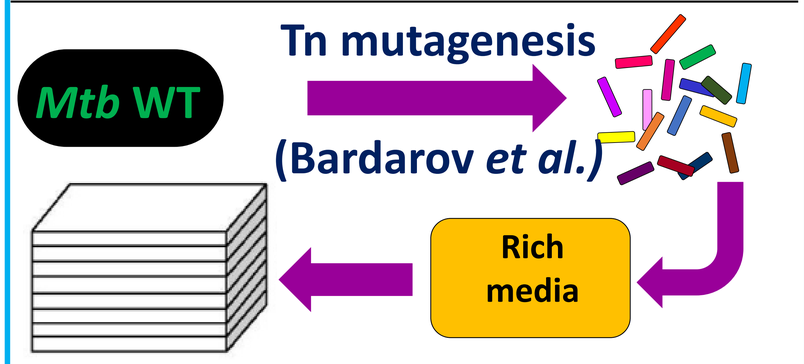
C-N Flux Analysis Continuous *Mtb* culture



JÜLICH Forschungszentrum

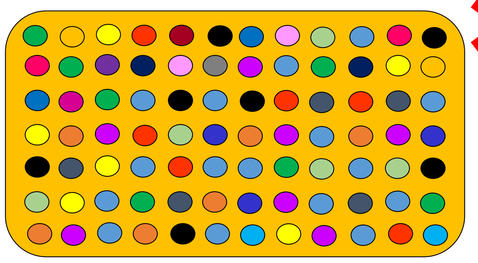
^{13}C-^{15}N Flux Analysis of *Mtb*

Essentiality N screen *In vitro Mtb*



11,000 spotted Tn-mutant library (in PBS)

Rich media	Roisin's + Urea
Roisin's + Glu	Roisin's + KNO_3
Roisin's + Asp	Roisin's + NH_4Cl



Essential genes

Tn-seq analysis

Identify auxotrophs

FUTURE PERSPECTIVES

- Using this system's strategy to study *Mtb* metabolism will help determine which N sources are required for *ex vivo Mtb* survival and the uptake and assimilation pathways involved to ultimately identify novel drug targets to treat TB

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