# Model-based Analysis of the Metabolic Effect of Citrate Stress on *Mycobacterium Tuberculosis*

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## Introduction

- *Mycobacterium tuberculosis (MTB)* is highly adaptive bacteria, it survives various stress conditions.
- It has been reported that macrophages when infected with *MTB* accumulate citrate in cytoplasm, which could influence the metabolic pathways of the bacteria.
- We hypothesize that citrate stress induces specific pathway adaptations in *MTB*, which can be identified through experimental data and genome-scale metabolic models. These adaptations may reveal potential metabolic targets for the treatment of drug-resistant MTB.

## Methods

### Genome-Scale Metabolic Models:

Cellular networks modeled by dynamic mass balance equations.

$$\frac{dx}{dt} = Nv(x)$$

Steady-state assumption

$$\frac{dx}{dt} = 0 \rightarrow Nv = 0$$

Model constraints

$$lb \leq v \leq ub$$

### Experimental Setup:

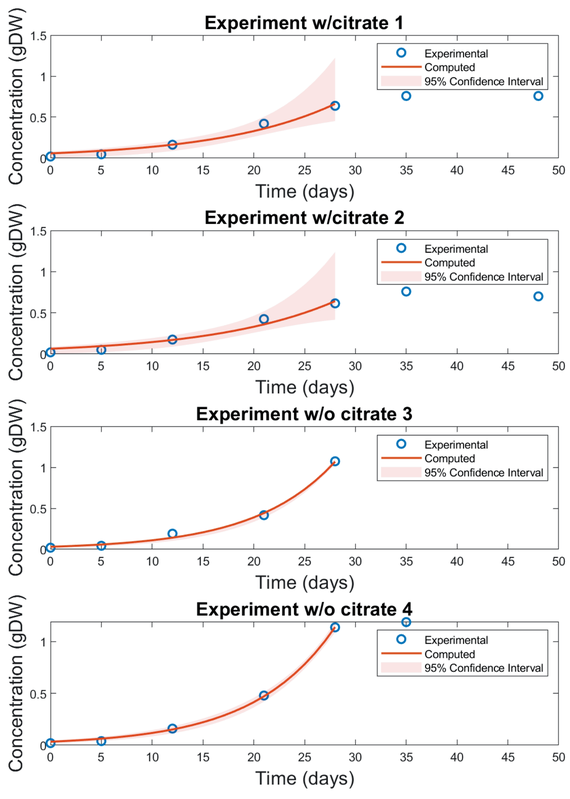
Experimental data was collected under two conditions: citrate with a replica and two without. Minimal growth medium with glycerol as carbon source was used.

| Experiment 1 Citrate | Experiment (Replica) 2 Citrate | Experiment 3 No citrate | Experiment (Replica) 4 No citrate |
|---|---|---|---|

Measured of growth kinetics and exometabolites concentrations over 48 days was performed on days 0, 5, 12, 21, 28, 35, and 48.

## Data Analysis

### Biomass curve fitting




- Mathematical model

$$B(t) = B_0 \times e^{\mu t}$$

- Model's constrains

$$\mu - 2\,SD \leq v_{biomass} \leq \mu + 2\,SD$$

The exponential growth phase occurs from day 0 through the 28th day. The presence of citrate appears to alter the growth pattern and lead to a decrease in growth.

Citrate can restrict essential ion availability to microorganisms, slowing growth. It may trigger stress responses or gene activation, altering cell metabolism and proliferation.

### Metabolites curve fitting

- Glycerol mathematical model

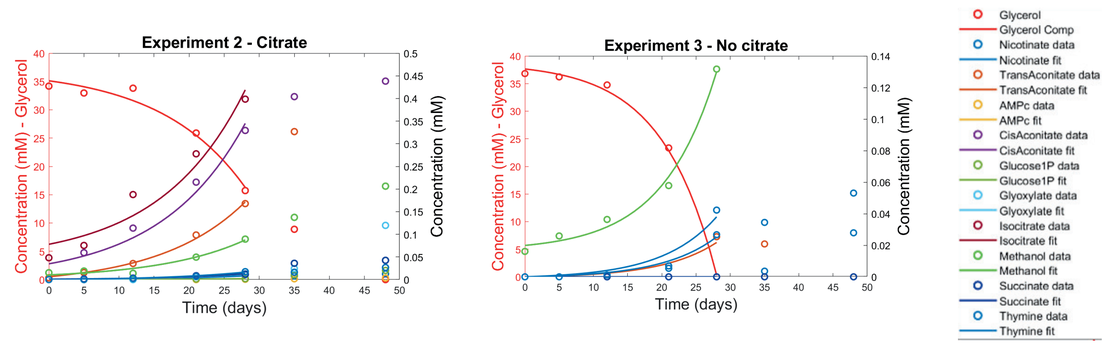
$$G(t) = G_0 - \left(\frac{B_0 \times r_{glc}}{\mu}\right) \times (e^{\mu} - 1)$$

- Produced metabolites mathematical model

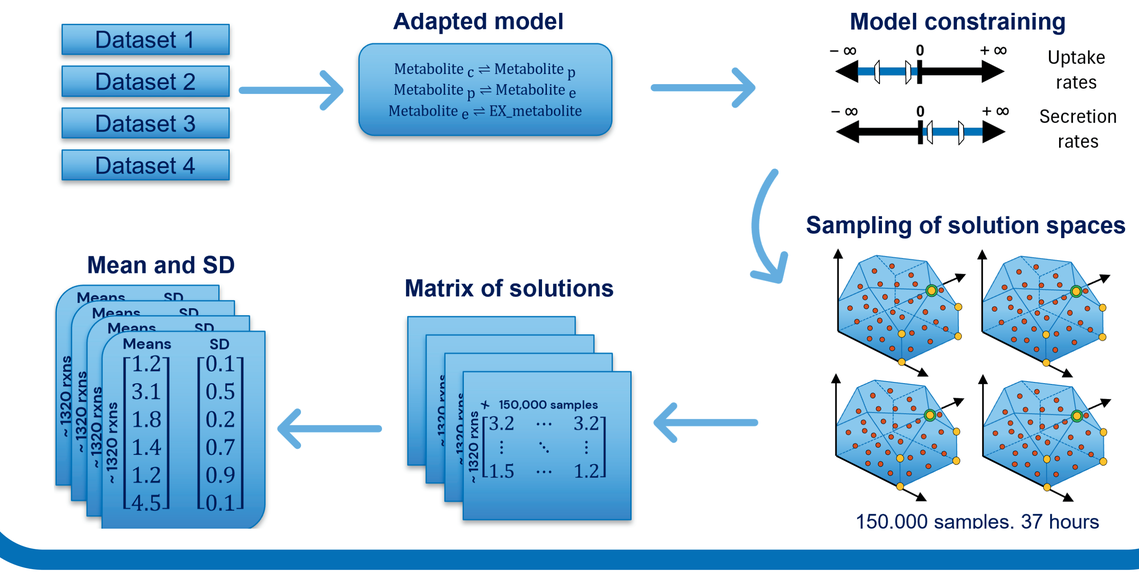
$$X(t) = X_0 + \left(\frac{B_0 \times r_{met}}{\mu}\right) \times (e^{\mu} - 1)$$

- Model constrains

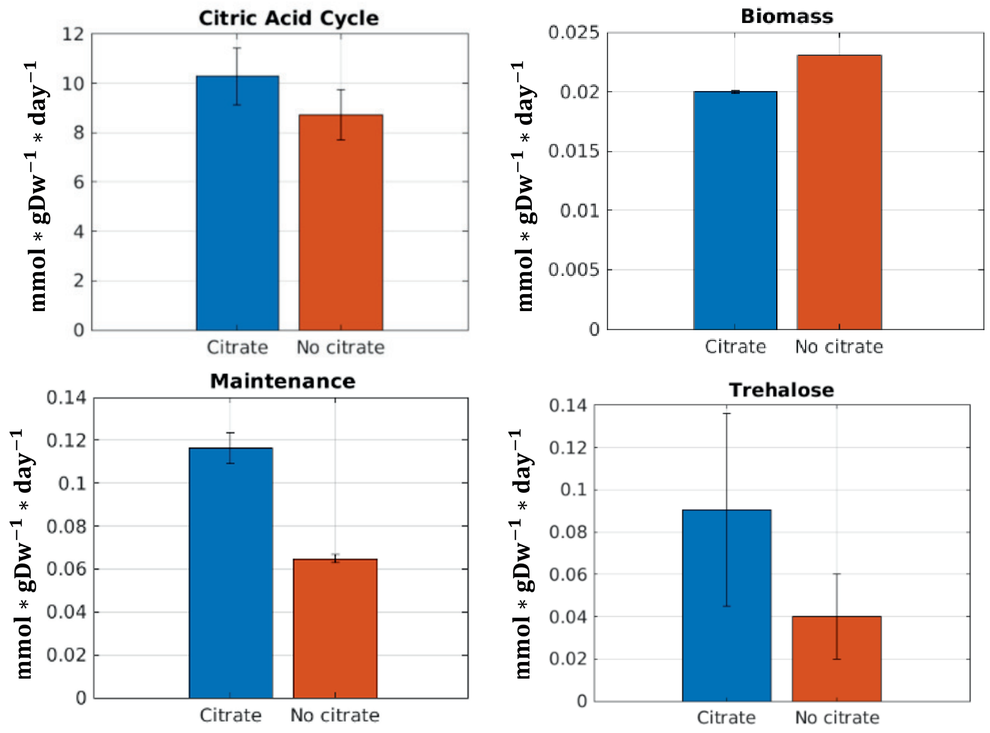
$$r_{met} - 2\,SD \leq v_{met} \leq r_{met} + 2\,SD$$




## Model constraining and sampling




## Results




*Since results between replicas were found very similar, only experiments 3 and 4 are compared

- Decrease in growth rate under citrate conditions is shown.
- TCA cycle and glyoxylate shunt under citrate stress is upregulated .
- Trehalose production is increased when citrate is present.

**Trehalose acts as precursor for the synthesis of cell wall glycolipids, is involved in the formation of the cell wall components critical for pathogenicity and the ability to evade the host immune response. Also helps MTB survive desiccation, freezing, starvation, and osmotic stress.**

**Conclusions :** Data was incorporated into a genome-scale metabolic model to investigate MTB's response to citrate stress and minimal growth medium conditions with glycerol as the carbon source. MTB appears to adjust its metabolism by enhancing gluconeogenesis, the TCA cycle, and the pentose phosphate pathways. This results in increased trehalose production, aiding the bacteria in survival and enhancing its virulence. Targeting these pathways could be a promising approach for developing drugs and tackle drug-resistant MTB strains.