

Stochastic Agent-Based Modeling of *Salmonella* Infections

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Introduction

Modeling host-pathogen interactions contributes to a deeper understanding of infectious processes. In our study, we examined macrophages infected with *Salmonella* using advanced image processing techniques, deep learning algorithms, and agent-based modeling.

Objective

Our goal was to measure bacterial incubation time, maximum bacterial load, the number of bacteria released during lysis, the division time of cells, and to quantify infection, death, and replication rates. Based on our experimental data, we developed a stochastic agent-based model to describe these processes. This model allows us to extrapolate features of the macrophage-bacteria system that are difficult to assess experimentally. Parameter optimization was performed using `pygad`, a genetic algorithm-based optimization library.

In addition to optimizing infection parameters, we also used `pygad` to estimate the maximum bacterial load that macrophages can tolerate before undergoing lysis. The simulation was implemented in `NetLogo`, allowing for easy and repeatable runs under various conditions.

Methods

We infected RAW 264.7 mouse-derived macrophage cell lines with *Salmonella Typhimurium* at low (MOI 10) and high (MOI 50) multiplicities of infection. The cell cultures were maintained in DMEM medium supplemented with 10% FBS. Macrophages were labeled with Cy5 and bacteria with TRITC fluorescent dyes. Data were acquired using a Nikon Eclipse Ti-E microscope in wells 8–21 of a 96-well plate, capturing 60 time points over 20 hours at 21 positions per well. These biological experiments were conducted during the Summer Undergraduate Research Program 2024 at NITMB, Northwestern University, in the Lane Lab under the guidance of PhD student Anika Marand and lab director Keara Lane.

Image processing was performed using the Cellpose segmentation algorithm. Using known infection, death, and division rates, along with lysis parameters, we constructed a delayed Gillespie-based stochastic agent model. Parameter fitting and bacterial load threshold estimation were achieved through `pygad` optimization. The mathematical modeling and simulation work was carried out at the University of Szeged, Hungary.

The delayed stochastic simulation algorithm (dSSA) modifies the traditional Gillespie formulation to account for reactions with a deterministic delay τ . The master equation is represented as:

$$\frac{dP(\vec{X}, t)}{dt} = \sum_{j=1}^M \left[a_j(\vec{X} - \vec{\nu}_j) P(\vec{X} - \vec{\nu}_j, t - \tau_j) - a_j(\vec{X}) P(\vec{X}, t) \right], \quad (1)$$

where $\vec{X}$ is the state vector, $\vec{v}_j$ is the state change vector for reaction j , a_j is the propensity function, and τ_j is the delay associated with reaction j .

Results

At MOI 10, infected cells appeared after approximately 420 minutes, while at MOI 50, infection was detectable by 200 minutes. Incubation time ranged between 240–540 minutes and was significantly influenced by cell size. Lysis occurred 200–300 minutes post-infection, once infected cells reached approximately 40% infection-to-cytoplasm pixel ratio.

The released bacteria occupied an average area of 45 pixels, corresponding to the release of around 40–50 bacteria into the extracellular space. Based on literature (Gog et al., 2012; Shi et al., 2016; Vlazaki et al., 2020; Ciupe and Heffernan, 2017; Cesar et al., 2022; Wang et al., 2023; Wrande et al., 2020), we set the cell death rate to $1.2 \times 10^{-3} \text{ s}^{-1}$, the division rate to $2.5 \times 10^{-4} \text{ s}^{-1}$, and the infection rate to $2.5\text{--}12.5 \times 10^{-5} \text{ s}^{-1}$.

Conclusion

Our results provide detailed insights into the behavior of macrophages infected with *Salmonella*. With the extracted parameters, we were able to construct a realistic stochastic infection model. The simulation was developed in NetLogo, enabling flexible and repeated experimentation. Our validated agent-based model may also serve as a simulation tool for infections in human-derived macrophages.

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