Bacterial filamentation: a bet for survival in stressful environments

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For Yihui Xie

Acknowledgements

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Abstract

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1-D, 2-D . . . One- or two-dimensional, referring **in this thesis** to spatial dimensions in an image.

Otter . . . . . One of the finest of water mammals.

Hedgehog . . . Quite a nice prickly friend.

Introduction

1

Experiment analysis

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1.1 Introduction

1.2 Experiment design

1.3 Exploratory Data Analysis

1.3.1 General preprocessing of data

The processing of the raw data consisted mainly of creating two levels of observation for the cells of both chromosomal strains and multicopy plasmids. The first level is at a cell granularity, that is, point properties. The second level consists of the cells over time, thus observing properties at the population level. We did this because it

would allow us to understand what factors are affecting filamentation and why.

We decided to normalize the fluorescence values of DS-Red and GFP for both experiments based on the values observed before the start of the experiment. It allowed us to have a basis to work with and compare the expressions between cells. In the case of DS-Red, we also applied a logarithmic transformation to observe subtle changes that would allow us to dig deeper.

Ultimately, we decided to classify cells into four fundamental groups based on whether the cell was filamented and survived (see 1.1). We define a *filamented cell* as a cell that is more than two standard deviations from the mean concerning the lengths observed before antibiotic entry into the system []. On the other hand, although there are multiple ways to define death in a cell context [], we decided to consider a *cell dead or missing* when we stopped having information about it, either because of its fluorescence levels or because it left the field of experimental tracking. Therefore, a *surviving cell* is one that existed before and after the exposure to the antibiotic.

1.3.2 Cells level

Figure 1.1: Cell classification and its distribution across experiments. We define a *filamented cell* as a cell whose length exceeded two standard deviations from the mean at any time during the experiment. A *surviving cell* is a cell that existed before and after exposure to the antibiotic. We removed from the analysis those cells that died before or were born after the exposure of the experiment. Therefore, we delimited the effect caused by the exposure to the antibiotic.

Figure 1.2: Cells life time classes distribution. Chromosomal cells appear to show more remarkable survival by the filamented class compared to non-filamented survivors. On the other hand, in cells with plasmids, the relationship seems to be the opposite. However, this may be due to several factors. For instance, the levels of antibiotic resistance inherent in a heterogeneous population is the most simple explanation. For each cell, we calculate its lifetime, that is, the time we saw the last cell minus the time we had our first observation of it. We truncated the time to one frame (*i.e.*, 10 minutes) after finishing the antibiotic exposure. From the first appearance of green or purple color (*i.e.*, surviving cells), higher lifetime values represent cells that existed long before the start of the experiment.

Figure 1.3: Cell's number of divisions. Chromosomal cells exhibited more divisions for surviving classes and non-surviving filamented cells (*i.e.*, purple, green, and red dots) relative to unchanged behavior in plasmid cells. Therefore, its contribution to filamentation remains uncertain.

Figure 1.4: Time since last division.

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Figure 1.21: Plasmid initial GFP survival probability.

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Figure 1.25: Population survivals binned by initial GFP over time.

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1.4 Discussion

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Models to the rescue; filamentation abstraction

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Scientists have extensively studied the mechanisms that orchestrate the growth and division of bacterial cells. Cells adapt their shape and dimensions in response to variations in the intracellular and extracellular environments by integrating information about the presence of nutrients or harmful agents in the decision to grow or divide. Filamentation is a process that occurs when rod-shaped cells stop dividing but continue to grow, thus producing elongated cells. Some cells can naturally grow as filamentous, while others only do so under stressful conditions. Here we use mathematical modeling and computational simulations to evaluate

a toxic agent's intracellular concentration as a function of cell length. We show that filamentation can act as a strategy that promotes the resilience of a bacterial population under stressful environmental conditions.

2.1 Introduction

By integrating information from the environment, cells can alter their cell cycle. For instance, some cells arrest the cell division in the presence of toxic agents but continue to grow. Previous studies have shown that this filamentation phenomenon provides a mechanism that enables cells to cope with stress, which leads to an increase in the probability of survival (Justice, Hunstad, Cegelski, et al. 2008). For example, filamentation can be a process capable of subverting innate defenses during urinary tract infection, facilitating the transition of additional rounds of intracellular bacterial community formation (Justice, Hunstad, Seed, et al. 2006).

Although filament growth can help mitigate environmental stress (e.g., by activating the SOS response system (Justice, Hunstad, Cegelski, et al. 2008)), the evolutionary benefits of producing elongated cells that do not divide are unclear. Here, we proposed a mathematical model based on ordinary differential equations that explicitly considers the concentration of intracellular toxin as a function of the cell's length (see Equation (2.1)). The model is built based on the growth ratio of measurements of the surface area (SA) and the cell volume (V), whereby the uptake rate of the toxin depends on the SA. However, $V's$ rate of change for SA is higher than SA for V, which results in a transient reduction in the intracellular toxin concentration (see Figure 2.1). Therefore, we hypothesized that this geometric interpretation of filamentation represents a biophysical defense line to increase the probability of a bacterial population's survival in response to stressful environments.

Figure 2.1: Cell dimensions relationship. We evaluated the resulting geometric properties on a grid of side lengths and radii with a pill-shaped cell. We can see that by maintaining a constant radius (typical case in bacteria such as *E. coli*) and increasing the side length, the surface area / volume relationship (SA/V) tends to decline since the V will grow at a higher rate than the SA.

2.2 Filamentation model

Let us assume the shape of cells is a cylinder with hemispherical ends. Based on this geometric structure, a nonlinear system of differential equations governing filamentation can be written as follows:

$$\begin{aligned} \frac{dT_{int}}{dt} &= T_{sa} \cdot (T_{ext}(t) - T_{vol}) - \alpha \cdot T_{ant} \cdot T_{int} \\ \frac{dL}{dt} &= \begin{cases} \beta \cdot L, & \text{if } T_{int} \geq T_{sos}, t \geq \tau_{sos} + \tau_{delay} \text{ and } L < L_{max} \\ 0, & \text{otherwise} \end{cases} \end{aligned} \tag{2.1}$$

It considers the internal toxin (T_{int}) and the cell length (L) as variables. T_{sa} and T_{vol} represent the surface area and volume of the toxin in the cell, respectively.

$T_{ext}(t)$ is a function that returns the amount of toxin in the cell medium. T_{anti} and α symbolize the amount of antitoxin and its efficiency rate, respectively. β as the rate of filamentation. L_{max} is the maximum size that the cell can reach when filamentation is on. T_{sos} and T_{kill} are thresholds for filamentation and death, respectively. Finally, τ_{delay} is the amount of time required to activate filamentation after reaching the T_{sos} threshold.

2.3 Results

2.3.1 Filamentation provides transient resistance under stressful conditions

When growing rod-shaped bacterial cells under constant conditions, the distribution of lengths and radii is narrow (Schaechter, Williamson, et al. 1962). However, when growing under stress conditions, some cells produce filaments (Schaechter, MaalOe, et al. 1958). This phenomenon may depend on the SOS response system (Bos et al. 2015), which can repair DNA damage, giving the cell greater chances of recovering and surviving under stress conditions. Besides, the SOS response has been reported to have precise temporal coordination in individual bacteria (Friedman et al. 2005). Among the stress conditions that can trigger the SOS response is exposure to beta-lactam antibiotics (Miller 2004).

In general, filamentation has been studied as an unavoidable consequence of stress. However, we considered filamentation an active process that produces the first line of defense against toxic agents. Therefore, a differential equation model was proposed that assesses the change in the amount of internal toxin as a function of cell length. At the core of this model, we include the intrinsic relationship between the surface area and the capsule volume since it is vital in determining cell size (Harris and Theriot 2016).

In figure 2.2, cells grow in a ramp-shaped external toxin signal without considering a toxin-antitoxin system. As time progresses, the toxin in the external environment increases, so the cell raises its internal toxin levels. At approximately

time 22, any cell reaches T_{sos}. The control cell (unable to filament) and the average cell (capable of filamenting) reach the death threshold, T_{kill}, at times 31 and 93 (hatched and solid black lines), respectively. Therefore, under this example, the cell has increased its life span three times more than in control by growing as a filament (green shaded area versus orange shaded area). In turn, figure 2.2 also shows stochastic simulations of the system in the intake of internal toxins. Considering that cell growth and death processes are inherently stochastic, stochastic simulations would be a better approximation. However, from now on, we will continue with the study of the deterministic model.

Figure 2.2: Effect of filamentation on intracellular toxin concentration. In the presence of an extracellular toxic agent, the intracellular concentration of the toxin (T_{int}) increases until reaching a damage threshold that triggers filamentation (T_{sos}, blue point), increasing cell length (L). When filamentation is on, the cell decreases T_{int} due to the intrinsic relationship between surface area and cell volume. When the cell reaches its maximum length, it eventually dies if the stressful stimulus is not removed (T_{kill}, red dot). The hatched line represents a cell that can not grow as filament. The orange shaded area is the time between stress and the non-filament cell's death, while the green shaded area represents the temporal gain for doing so. The blue background lines represent stochastic simulations of the same system.

2.3.2 Filamentation increases the minimum inhibitory concentration

Antimicrobial resistance (AMR) can be considered one of the most critical health problems of the century. That is, microorganisms' ability to grow despite exposure to substances designed to inhibit their growth or kill them. In April 2014, the World Health Organization (WHO) published its first global report on AMR surveillance ("Editorial Board" 2014). Taking out of the darkness a common fear, a possible post-antibiotic future in which common infections or minor injuries can kill. Therefore, understanding the mechanisms of avoiding antibiotic action is essential for producing knowledge and developing strategies that reduce the generation of resistant bacteria.

A classic experiment in laboratories finds the concentration that inhibits bacterial growth through exposure to different toxin doses. The concentration found is known as the minimum inhibitory concentration (MIC) (Andrews 2002). Bacteria are capable of modifying their MIC through various mechanisms, for example, mutations (Lambert 2005), impaired membrane permeability (Sato and Nakae 1991), flux pumps (Webber 2003), toxin-inactivating enzymes (Wright 2005), and plasticity phenotypic (Justice, Hunstad, Cegelski, et al. 2008). The latter is our phenomenon of interest because it considers the change in shape and size, allowing us to study it as a strategy to promote bacterial survival.

We decided to analyze the MIC change caused by filamentation through stable exposure experiments of different toxin amounts at other exposure times. Computational simulations show that when comparing cells unable to filament with those that can, there is an increase in the capacity to tolerate more generous amounts of toxin, increasing their MIC (see Figure 2.3). Therefore, it confers a gradual increase in resistance beyond filamentation's role in improving the cell's life span as the exposure time is longer.

Figure 2.3: Effect of filamentation on minimum inhibitory concentration (MIC). By exposing a cell to different toxin concentrations with stable signals, the cell achieves a set MIC for conditions without or with filamentation (separation between stressed and dead state) for each exposure time, without representing a change for the normal state cells' points (blue zone). Thus, the green line represents a gradual MIC increase when comparing each MIC between conditions for each exposure time.

2.3.3 Heterogeneity in the toxin-antitoxin system represents a double-edged sword in survival probability

One of the SOS response system properties is that it presents synchronous activation times within homogeneous populations (Friedman et al. 2005). It has constant gene expression rates that help it cope with stress; however, it is possible to introduce variability by considering having multicopy resistance plasmids (Million-Weaver and Camps 2014). Therefore, the response times would have an asynchronous behavior at the global level but synchronous at the local level.

To include this observation into the model, we include a negative term to the internal toxin representing a toxin-antitoxin system. Therefore, the model now has an efficiency rate of the antitoxin and a stable amount per cell. We simulate

the effect of the toxin-antitoxin system variation within a 1000 cell population; we initialize each one with similar initial conditions, except for the amount of internal antitoxin, defined as $T_{anti} \sim N(\mu, \sigma)$. Considering that T_{anti} values < 0 are equal to 0. For each experiment,$\mu = 25$, while it was evaluated in the range $[0 - 20]$. For the generation of pseudo-random numbers and to ensure the results' reproducibility, the number 42 was considered seed.

As shown in Figure 2.4, when we compare heterogeneous populations in their toxin-antitoxin system, we can achieve different population dynamics, that is, changes in the final proportions of cell states; normal, stressed, and dead. This difference is because the cell sometimes has more or less antitoxin to handle the incoming stress situation.

Figure 2.4: Variability in the toxin-antitoxin system produces different proportions of cell states. Histograms represent the distribution of antitoxin quantity, while the curves represent the population's fraction over time. The cell will start to filament after reaching a certain internal toxin threshold, T_{sos}. Therefore, the expected global effect on the population's response times based on the amount of antitoxin is asynchronous, while at the local level, it is synchronous. Consequently, different proportions are presented in the cellular states since some cells will activate the filamentation system before and others later.

2. Models to the rescue; filamentation abstraction

Considering that the toxin-antitoxin system's variability can modify the proportions of final cell states, a question arose about heterogeneity levels' global behavior. To answer this question, we evaluate the probability of survival for each population, defined by its distribution of antitoxin levels. In this way, we characterized the population survival probability function into three essential points according to its effect: negative, invariant, and positive (see Figure 2.5). Furthermore, these points are relative to the homogeneous population's death time in question (τ_{kill}): when $t < \tau_{kill}$ will represent a detrimental effect on survival, $t = \tau_{kill}$ will be independent of variability, and $t > \tau_{kill}$ will be a beneficial point for survival. Therefore, we concluded that the effect of heterogeneity in a toxin-antitoxin system represents a double-edged sword.

Figure 2.5: Effect of variability on the toxin-antitoxin system. The color of the heatmap is representative of the fraction of living cells at exposure time. The white vertical line represents the death time of the homogeneous population (τ_{kill}). At $t < \tau_{kill}$, it is shown that the fraction of survivors decreases as the variability in the population increases. When $t = \tau_{kill}$, the variability does not affect the fraction of survivors, but it represents a percentage improvement for the homogeneous population. Finally, when $t > \tau_{kill}$, the heterogeneity of the population favors survival.

2.4 Discussion

Today, there have been advancements in the number of techniques that have allowed it to extend quantitative analyses to individual cells' dynamic observations (Campos et al. 2014; Meldrum 2005; Sliusarenko et al. 2011; Taheri-Araghi et al. 2017; Ursell et al. 2017). Therefore, studying their cellular behavior every day from medium to medium can be somewhat reproducible, facilitating the association of complex biological functions in simple mathematical equations (Neidhardt 1999).

Here, we proposed a mathematical model showing that filamentation could serve as a population's resilience mechanism to stress conditions. Finding that filamentation's net effect generates an additional window of time for the cell to survive, decreasing the toxin's intracellular concentration. However, we also found that a side effect of filamentation is to increase the cell's minimum inhibitory concentration. On the other hand, when we introduce variability in the amount of antitoxin in a cell population, we found that heterogeneity can be a double-edged sword, sometimes detrimental and sometimes beneficial, depending on the time of exposure to the toxic agent.

Due to the above, despite being simple, the model could have the ability to recapitulate the behavior seen in nature from variables that we can calculate easily with single-cell measurements. However, in other situations, it could be helpful to consider the addition of variables such as cell wall production and peptidoglycans' accumulation, among others. Notwithstanding the lack of parameters that are a little closer to reality, confirming that the model can work under experimental conditions would represent an achievement due to its explanatory simplicity, starting, in this way, the path of the study of filamentation oriented to the ecology of stress.

3
Discussion

Appendices

A

The First Appendix

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