Bacterial filamentation: a bet for survival in stressful environments

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Welcome

Abstract

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 (Wang et al. 2014; Wang, Yin, and Chen 2014; Jaimes-Lizcano, Hunn, and Papadopoulos 2014; Sheryl S. Justice et al. 2008). Some cells can naturally grow as filamentous, while others only do so under stressful conditions (Cayron, Dedieu, and Lesterlin 2020; S. S. Justice et al. 2006). 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.

Acknowledgements

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Introduction

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.

Bacterial adaptability to hostile environmental conditions can be explained by different elements, not necessarily exclusive. For instance, mutational phenomena that allow bacteria to evade the mechanisms of action of certain antibiotics have been one of the most studied (Dever and Dermody 1991; Andersson 2005). However, the continuous technological development has allowed us to explore hypotheses where phenotypic heterogeneity is considered in detail, allowing us to study emergent behaviors in isogenic populations (Ackermann 2015). Thus, we have gone from studying bacterial communities as a whole to studying them from each of the cells that compose them and their emergent properties.

Single-cell microfluidics is one of the technologies that has made it possible to create and maintain the microenvironments necessary for studying bacteria (Yin and Marshall 2012). Among the most outstanding utilities of microfluidics, we can find the engineering of bacterial systems, microbial ecology, bacterial cell cycle, homeostasis, even cell shape, and geometry. The latter is one of the characteristics that allow the study of bacterial filamentation, a phenomenon that occurs when the cell stops dividing but continues to grow, thus producing elongated cells in the form of filaments.

Mathematical modeling is among the most common strategies to address the AMR problem. Mathematical modeling allows to pose real-life problems in a space filled with mathematical language, solve them, and test their solutions in a real-life living system (Verschaffel, Greer, and Corte 2002). Therefore, this approach can also be used to analyze in detail why a particular biological phenomenon is occurring, how its behavior can be modified, and, finally, to design specific experiments to determine their accuracy and usefulness.

This thesis describes and discusses how and why bacterial filamentation may be a general mechanism for cell survival upon exposure to toxic agents, such as antibiotics, based on experimental analyses and mathematical modeling. We divided this thesis into three chapters that

explain the methodologies used and take us one step closer to understanding filamentation with each chapter.

Chapter @ref(image-processing) describes the fundamental process to identify and quantify the properties of each cell over time, for example, its length, the amount of internal toxin, and the amount of resistance to the toxin.

Chapter @ref(experiment-analysis) used the data processed in the previous chapter to explore bacterial filamentation at the population and single-cell level. Data exploration allowed us to simultaneously observe the behavior of filamentation and its properties in heterogeneous populations. For reference, one population with an antibiotic resistance gene located on the chromosome and another on multicopy plasmids.

Finally, in chapter @ref(model-analysis), we postulated a mathematical model that considers the relationship of cell surface area and volume to the uptake of a toxic agent diffusing into the medium. This model allowed us to specifically evaluate the effect of filamentation in an environment similar to that observed experimentally. Thus, experiments and models work together to learn more about a biological phenomenon to help understand and combat the AMR problem.

1 Image processing

1.1 Introduction

With the progress of technology, optical and fluorescence microscopy has become a fundamental tool for the characterization and understanding of the bacterial world. Microscopy has allowed humanity to extend its senses to observe the unknown world with exciting new perspectives that they might never otherwise have envisioned. Furthermore, microscopy offers a clear advantage over other techniques used to characterize bacteria since it can acquire data from living cells in spatial resolution (Schermelleh et al. 2019).

Including the discovery of fluorescent proteins (*e.g.*, GFP and DsRed) and improvements in fluorescent reporters, it is possible to specifically label specific cellular components and track cellular functions (Specht, Braselmann, and Palmer 2017). On the other hand, mechanical and intellectual development of microfluidic research techniques provides an excellent opportunity to overcome bio-medical and chemical techniques (Convery and Gadegaard 2019). Collectively, it is possible to study communities of bacteria at the level of individual cells (Balaban et al. 2004; Elowitz et al. 2002).

Although all this technological development has provided a significant advance for the scientific community, after acquiring fluorescence images, the extraction of quantitative properties from these images is crucial, but unfortunately, a difficult step for analyzing experiments. Not so long ago, image analysis in biology relied on manual quantification. However, manual analysis suffers from two main problems: 1) accuracy and 2) scalability (that is, analyzing miles or more images). Fortunately, improvements in image accuracy and computational image analysis capabilities are revolutionizing the quantification of biological processes through (Caicedo et al. 2017; Smith et al. 2018). Therefore, the manual correction required to analyze the experiments is minimal.

Here, we used a series of programs in μJ (https://github.com/ccg-esb-lab/uJ), which consists of an ImageJ macro library (mainly) for quantifying unicellular bacterial dynamics in microfluidic devices (Schneider, Rasband, and Eliceiri 2012). The specific steps used are described below and are summarized in Figure (**ref?**)(fig:).

1.2 Preprocessing

We exported the figures obtained by the NIS-Elements software (RRID:SCR_014329) from the microfluidics experiments in TIFF (Tagged Image File Format) format. Each figure was named as follows: *experimentxyc1t001* where *experiment* indicates the name assigned to the experiment, *xy* the trap number, *c* the fluorescence channel, and *t* the passage of time.

Subsequently, we compile the images, rename them and save them as images in different folders. We maintained the classification by fluorescence channels and phase contrast, and within the channel folder, it is the sub-classification by trap number.

1.3 Segmentation

To determine which parts of the photographs correspond to cells, we carry out an image segmentation analysis. Segmentation consists of classification at the pixel level, which allows us to define the pixels that give identity to the limit of a cell, its interior, and the image's background (everything that is not a cell). A new image is generated from the above, known as the segmentation mask, containing only the pixels that identify cells.

To build the segmentation mask, we used *Deepcell* (Van Valen et al. 2016). *Deepcell* is a network trained with a robust set of images that people previously classified as cells. However, the generation of the segmentation masks is not absolved of errors (see also Section @ref(manual-corrections)). Sometimes we must correct them manually due to 1) mistakenly identifying two or more cells as one, 2) identifying two or more cells when it is only one cell, and 3) failing to identify a cell.

1.4 Tracking

From the image segmentation, we obtain ROI files (region of interest), which contain coordinates of the position of individual cells in each photograph (Brinkmann 2008). Tracking is the tracking of a region of interest in a consecutive series of images. In this case, the tracking generates the identification of the lineages, that is, the ancestry of each cell.

We read the ROI files in Python through the *shapely* package, which efficiently reconstructs polygons, thus calculating the length of the cells (**10.5555/1593511?**; **shapely2007?**). Also, in Python using ROI files, we track cells with the k-nearest neighbors algorithm that uses various properties such as fluorescence intensity, length, and shape of each cell, to identify cell lineages (Altman 1992).

1.5 Manual corrections

For cell-tracking manual correction, we used *Napari,* an open-source python-based tool designed to explore, annotate, and analyze large multidimensional images (**sofroniew2021a?**). Our custom cell-viewer allows us to easy lineage data visualization, custom-plotting, and lineage-correction. Code for our cell-viewer is available on https://github.com/ccg-esb-lab/uJ/tree/master/single-channel.

We produced high-throughput data of thousands of cells with a single-cell resolution to the end of the lineages manual reconstruction. We obtained data about time-series of fluorescent intensity, morphological properties of individual cells (*e.g.*, elongation, duplication rate), and time-resolved population-level statistics (*e.g.*, probability of survival to the antibiotic shock).

1.6 Data extraction

We construct a file in columnar format through image processing that contains the information necessary to analyze each experiment (*i.e.*, chromosomal and plasmids) in its different traps (*i.e.*, XY identifier). See Table 1.1 for a full description of the output data. Subsequently, the table was analyzed in R for statistical computation and plotting (see Chapter @ref(experiment-analysis)) (**R-base?**).

Table 1.1: Resulting table from image processing.

Column	Description
experimentID	Unique identifier of the experiment.
trapID	Unique identifier of the trap used.
lineageID	Unique integer of the stem cell and its ancestry.
cellID	Unique identification number for each cell existing since the beginning of the experiment or generated later.
motherID	Represents the identification number of the stem cell that gave rise to the progeny.
trackID	Indicates the x-y coordinates where the cell being tracked starts.
roiID	Indicates the x-y position in which the cell is located, followed after each photograph.
frame	Number of the photograph in the sequence of photographs taken, indicating the elapsed time (10 minutes per frame).
length	Cell length.
division	Indicates cell division events, represented by the value 1 when they occur and 0 otherwise.

Column	Description
GFP	Represents the relative fluorescence intensity in each cell by green fluorescent protein (*i.e.*, GFP).
DsRed	Represents the relative fluorescence intensity for cells generated by rhodamine's internalization (*i.e.*, DsRed); an indicator of cell death events.
tracking_score	Determine how good or bad the tracking of a cell was.
state	Indicates the state of the cell determined from its length and fluorescence thresholds. -1 for death, 0 for normal, and 1 for filamentation (see Section @ref(experiment-general-preprocessing) for detailed information).

2 Experiment analysis

3 Models to the rescue; filamentation abstraction

4 Discussion

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