Representing Bacterial Growth Data Obtained Through Kinetic Assay

Tatiana McGarry

R Tutorial Overview

Research Methods in Biology 2

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Introduction

Antimicrobial peptides (AMPs) have emerged as promising candidates for combating bacterial infections. Recently, AMPs fitted with probes specifically targeting certain microorganisms have also gained attention, due to their ability to selectively inhibit the pathogen of interest and leave other microbes largely unaffected. Assessing the efficacy of both targeted and un-targeted AMPs against bacterial pathogens necessitates robust analytical methods to derive meaningful insights from large datasets generated during growth assays. In the model presented here, two important questions are addressed through the evaluation of experimental data: 1) How does exposure to varying concentrations of an antimicrobial peptide affect the growth of a target pathogen?, and 2) Does a targeted version of an AMP succeed in inhibiting or eliminating the bacterial strain of interest to the same degree as its non-targeted counterpart?

Methods

The experimental setup involves first preparing a 96 well dilution plate with the target pathogen cultured along with varying concentrations of two antimicrobial peptides (for the sake of data confidentiality, these have been anonymized as AMP1 and AMP2), along with both positive and negative controls (positive controls contain bacteria and no AMP; negative controls contain only medium). Concentrations of AMPs range from 0.5 to 32 μM. There are two AMPs tested per plate, and these are further partitioned into guided and unguided versions of each. There are replicates for both controls (6 positive and 6 negative) and for each of the experimental conditions (3 each). A plate thus prepared is then left to incubate in a portable optical density (OD) reader for periods typically ranging from 24 to 80 hours (and sometimes more). Optical density is is a measure of the transmission of light through a medium, and is frequently used in microbiological experiments to estimate the density of cells in liquid culture [8] (typically, wells containing only medium are used as a "blank" reference during machine calibration so that the initial turbidity of the medium is not included in the measurement).

The portable OD device records optical density readings of each well at predetermined intervals (e.g. every 30 minutes). Every well in the plate becomes a column in a .csv file that is downloaded by the user at the end of the experiment. Additionally, a column is used to represent the times at which readings were taken, which means the final .csv file will have 97 columns. Each row of the file represents one time interval during which readings were taken, so if the device is programmed to take readings every 30 minutes for 80 hours, this would result in 96 * 161 = 15,456 separate optical density readings that need to be parsed and interpreted. With such a large quantity of data, it is necessary to employ a systematic approach to extract meaningful growth curves that can be used to visualize the results of the experiment. The attached code provides a way to accomplish this in R.

Acknowledgement and Notes about Code Approach

Much of this code was written to parse Cerillo Stratus optical density output for a rotation project, during which I worked with Dr. Ankan Choudhury in the Greathouse Lab. Before I began the project, Dr. Choudhury had already developed a similar approach using R, which I assisted in refining and later referenced during my work on my own code. Since then, I have totally rewritten the code for my own purposes and changed almost everything (besides certain things that are part of standard practice and don't vary much). However, my work was still informed by using that existing approach as a reference, so I want to acknowledge that.

In particular, the general visual layout of the plot is not something I designed, but which I modeled after that reference (I am not speaking of the code, which I did write, but about the visual organization of the plot into two faceted rows of optical density readings). In my own version I used different visual elements, including different geometries and fonts than the original, along with a custom color palette. I tested different layouts as well, but in the end I decided to leave it the way it was done by Dr. Choudhury, because I think it is the best way to organize the data.

Selected References

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