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Gulinigeer Nurikemu December 8, 2017

Temporal Dynamics of Appearance of Bacterial Colonies on Solid Surfaces

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Abstract

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By observing and characterizing bacterial colony appearances on both antibiotic-free nutrient solid surfaces and surfaces with sub-lethal level antibiotics, we found that the colony appearance under the former condition follows the deterministic model of growth due to the fact that majority of colonies appeared at the same time and the spread of appearances was relatively narrow. However, the results from the latter condition can best be explained by the stochastic model since the colony appearance in this case displayed a wide spread that can be understood only after taking probability elements into consideration. This growth under the influence of sub-lethal level antibiotics was then studied in comparison to the known master equation of the stochastic model: $\frac{dP_n(\mathrm{t})}{dt} = \lambda(n-1)P_{n-1} - (\lambda+\mu)nP_n + \mu(n+1)P_{n+1}$, and the model was fit to the data by setting parameter n (population size of a colony) to 10^4 and by inputting specific values into the birth rate λ and death rate μ.

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Introduction

Most types of bacteria are able to grow and survive both in liquids and on solid surfaces. Therefore, in laboratory studies, there are also two in vitro methods to culture bacteria: 1. using liquid nutritious broth medium in test tubes, 2. using solid medium (agar plus nutrients) that is spread evenly on a Petri dish (called "plating"). Practical questions in the field of medicine or environmental sciences, such as eradication of harmful bacteria using antibiotics, cannot be solved without a sufficient understanding of bacterial growth since antibiotic susceptibility is closely associated with the growth [1]. Therefore, in this research, we studied bacterial growth on nutritious solid surfaces with *Escherichia coli* (often abbreviated as *E. coli)* being our model organism. *E. coli* is commonly found in intestines of many organisms, and it is the most widely used model bacteria in the field of microbiology. Basic studies of wild type *E. coli* (meaning the non-mutant type that occurs in nature) under the influence of various antibiotics, although performed in vitro, will still shed light on in vivo behavior of bacteria and their interaction with antibiotics.

After transferred from liquid growth environments to nutritious solid surfaces, which in our case were comprised of Luria-Bertani (often abbreviated as LB) medium plus agar, each bacterial cell starts to double after a certain period of dormancy (called "lag phase"). After the number of offspring reaches a certain size of n, a colony that is large enough to be observed by the naked eye will be formed on the surface. Observing and characterizing the appearance of colonies with respect to time is believed to have important significance in understanding the growth pattern of bacteria on solid surfaces. This type of temporal studies on colony appearance on solid surfaces have been conducted before, for instance the 1984 study of Ishikura and Hattori explores the same topic [2]. However, their study was directed toward environmental microbiology, hence their interest was in growth at room temperature of 27 ℃. In our case, we always deal with human body temperature of 37 ℃, which is significantly higher than the room temperature, and it is very likely that this discrepancy leads to different results.

As it has been briefly discussed previously, a colony on a plate originates with a single bacterial cell. Each parent cell doubles with a certain division rate r, which is the number of division per unit time. If the initial number of cells per unit volume (cell concertation) is N_0 then after one division, the new total number of cells per unit volume N_1 is simply

$$N_1 = N_0 \cdot 2$$

after d divisions, the final concentration N_n will be

$$N_n = N_0 \cdot 2^d$$

If the total duration of division is t, then N_n should be expressed as

$$N_n = N_0 \cdot 2^{r(t)}$$ [3].

This deterministic description is one of the simplest mathematical models in population dynamics. The continuity of time here results in an unrealistic assumption that the size of the population is also continuous. This continuum description of population dynamics (also known

as “deterministic description”), is less comprehensive and less accurate while compared to its counterpart, stochastic description. By contrast, the state space of the corresponding stochastic process is discrete (the counts of individuals are discrete by definition), and the final population size n at time t is described by a set of probabilities P_n(t). The discreteness of the stochastic model that is demonstrated as discreet jumps between different states is well illustrated in Figure 1 [4].

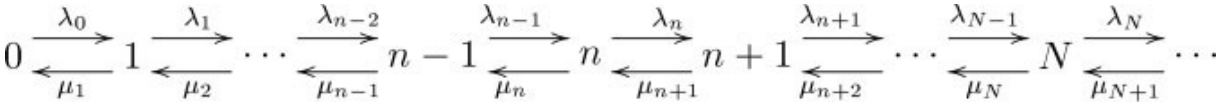

Figure 1: A jump to the right represents a birth process happens in a small time period of Δt, and the probability of this event is $\lambda \Delta t$, where birth rate λ is the number of birth per unit time. Similarly, a jump to the left is a death process with a death rate of μ.

The stochastic model for a population with a birth rate of λ (number of birth per unit time) and a death rate of μ (number of death per unit time) is given as [5]:

$$\frac{dP_n(\mathrm{t})}{dt} = \lambda(n-1)P_{n-1} - (\lambda+\mu)nP_n + \mu(n+1)P_{n+1}.$$

The term on the left is the rate of change of the probability of achieving a population size of n at time t. The first term on the right of equation represents the probability that the population size increases from n-1 to n (this is why it includes the birthrate λ); similarly, the third term is the probability that the population size decreases from n+1 to n. The middle term represents the probability that the size remains as n. If we assume the solution of this partial differential equation is in the form of $P_n = Aa^{n-1}$, then this yields

$$A(t) = \mathcal{E}(t)\,(\tfrac{1-\frac{\mu}{\lambda}}{\mathcal{E}(t)-\frac{\mu}{\lambda}})^2, \qquad a(t) = \tfrac{\mathcal{E}(t)-1}{\mathcal{E}(t)-\mu/\lambda}, \qquad \mathcal{E}(t) \equiv e^{(\lambda-\mu)t}.$$

After substitution, we get

$$P_n(t) = e^{(\lambda-\mu)t}\,(\tfrac{1-\frac{\mu}{\lambda}}{e^{(\lambda-\mu)t}-\frac{\mu}{\lambda}})^2\,(\tfrac{e^{(\lambda-\mu)t}-1}{e^{(\lambda-\mu)t}-\frac{\mu}{\lambda}})^{n-1} \quad (1)$$

, We plotted this equation for various values of n. This results in different probability distributions with shifted peaks and various spreads (Figure 2). As it’s been briefly mentioned previously, the stochastic model is fundamentally different from the deterministic model since the latter does not involve any element of probability distribution. Therefore, the graph of a deterministic model would be a delta-function like figure with only one sharp peak (Figure 3). Applying these two models to our study, it can be implied that if cells in each colony divide in a deterministic fashion, then we should expect all colonies to reach the expected, naked-eye-visible size of n at the same time. In other hand, if cell division is governed by the stochastic model, then the appearance of colonies will be spread into a wide range of time due to the involvement of probability, instead of being a population-boom-like sharp peak.

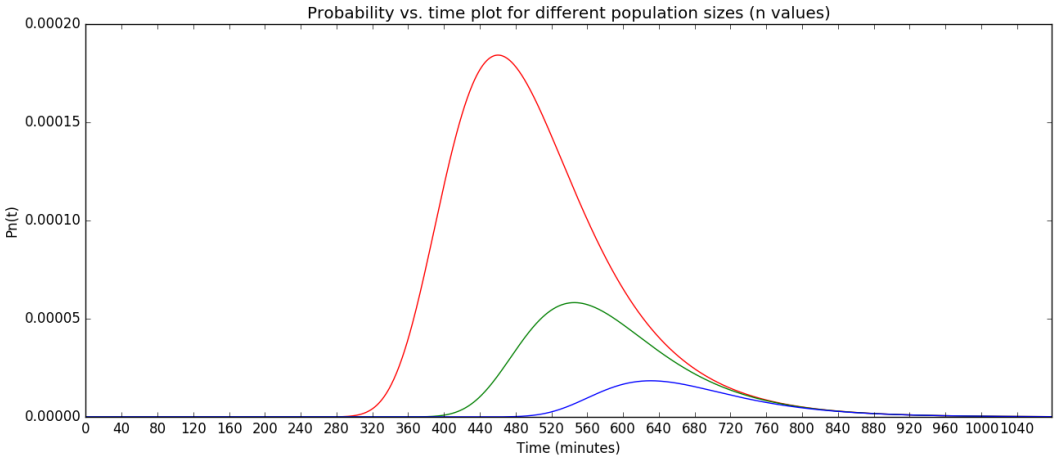

Figure 2. Stochastic model: red, green, and blue curves represent population size n of $10^3, 10^{3.5},$ and 10^4 respectively.

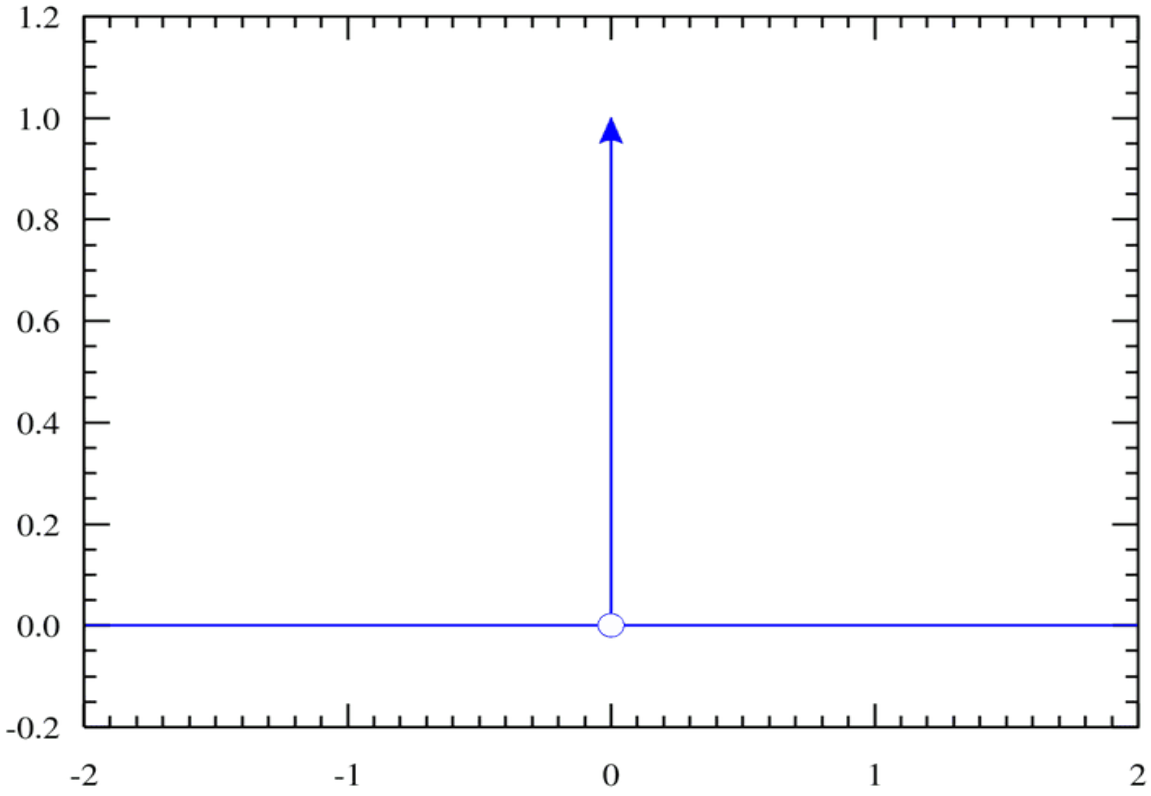

Figure 3. Deterministic model can be illustrated by a delta-function-like sharp peak.

These two models were then compared to the pattern of appearance of wild type *E. coli* colonies on both antibiotics-free and with-antibiotics plates to examine appearance patterns

agree with which of these two models. The detailed information about experimental set-ups will be discussed in the next section.

Experiment

Culture preparation. An average-size colony of wild type *E. coli* NMK1 strain on a stock LB plate stored at 4 ℃ was picked by a inoculation loop and inoculated into 2 ml of LB Broth (Miller) medium (seed culture). After 4-5 hours of growth, the optical density (OD_{600}) of the culture would be around 5, which means the growth has reached the stationary phase (growth rate equals 0). A certain volume of inoculum was then transferred to the medium (pre-culture) that comprises 2 ml of LB broth with 10 uM of glucose (for the purpose of ample carbon source) to set the initial cell OD to be 0.001. After the pre-culture density reached the stationary phase of OD of 5, the previous inoculation was repeated to set the starting OD of the experimental culture at 0.001.

Plate Preparation. Two plates for the control group were made by pouring 40 ml of autoclaved LB Agar (Miller) into two Petri dishes. 4 or 6 (depending on the specific task of that day) plates for 2 or 3 experimental groups (two replicas in each group) with designated antibiotic types and concentrations were made by pouring respective LB Agar plus antibiotic solutions contained in designated autoclave flasks. Plate preparation was finished about an hour into the start of the experimental culture, and the plates were incubated at 37℃ until the experimental culture reaches the desired OD. This stay was approximately two hours long, since it took in total 3 hours for the experimental culture to reach the designated platting OD of 0.4. The reason we plated at OD of 0.4 was because this is within the range of exponential growth phase in which the growth rate is constant; thus, this phase is considered as a steady state where all properties of cells are constant [3]. 300 ul of experimental culture at OD of 0.4 was removed from the culture tube and immediately diluted serially in 11.6 g/L NaCl solution to 10^{-5} folds. 150 ul of final diluted solution was plated on all experimental and control plates. Plates were then transferred to and kept in an incubator at 37℃ until the observation takes place.

Design of Observation. Naked eye observation was conducted at every 40 minutes, and the number of newly emerged colonies were recorded with respect to specific time points. In order to guarantee the consistency of counting, all experiments were conducted under same lighting condition and by the same experimenter. We made sure the counting takes as little time as possible, and after finishing counting, the plates were returned back to the incubator immediately to prevent any negative effects of sudden temperature change.

Results and discussions

As it is shown in Figure 4, on all four control plates with no antibiotics, colonies became visible at 320 minutes, and the spread of appearance was relatively narrow with a range of 320

to 560 minutes. In contrast, on experimental plates with cefsulodin that yielded 80% survival, colonies appeared later than the control group, and kept emerging up until 920 minutes (Figure 5). The subsequent two sets of experiments with a different concentration of cefsulodin and ofloxacin also displayed a lack of sharp peaks and a wide spread of colony appearances (Figure 6 and 7).

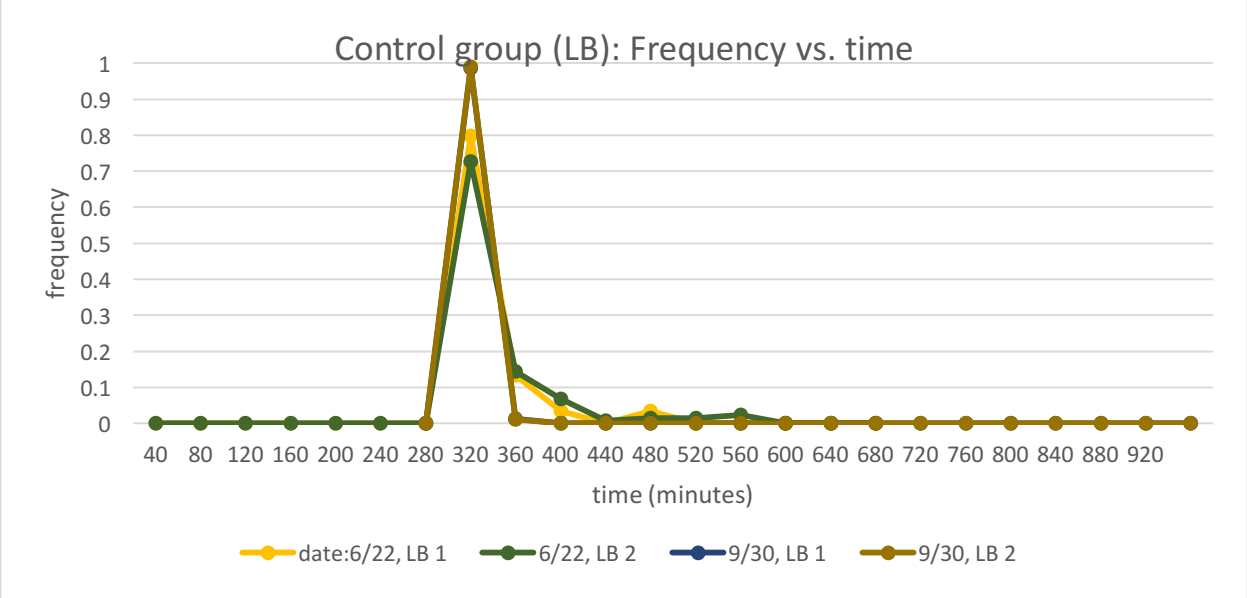

Figure 4. On control plates where there was no antibiotics, majority of colonies appeared at the same time, and the spread of appearance was relatively narrow. Note that the y-axis "frequency" was simply equal to the ratio of number of colonies appeared at that specific time point to the total number of colony formed eventually on that plate.

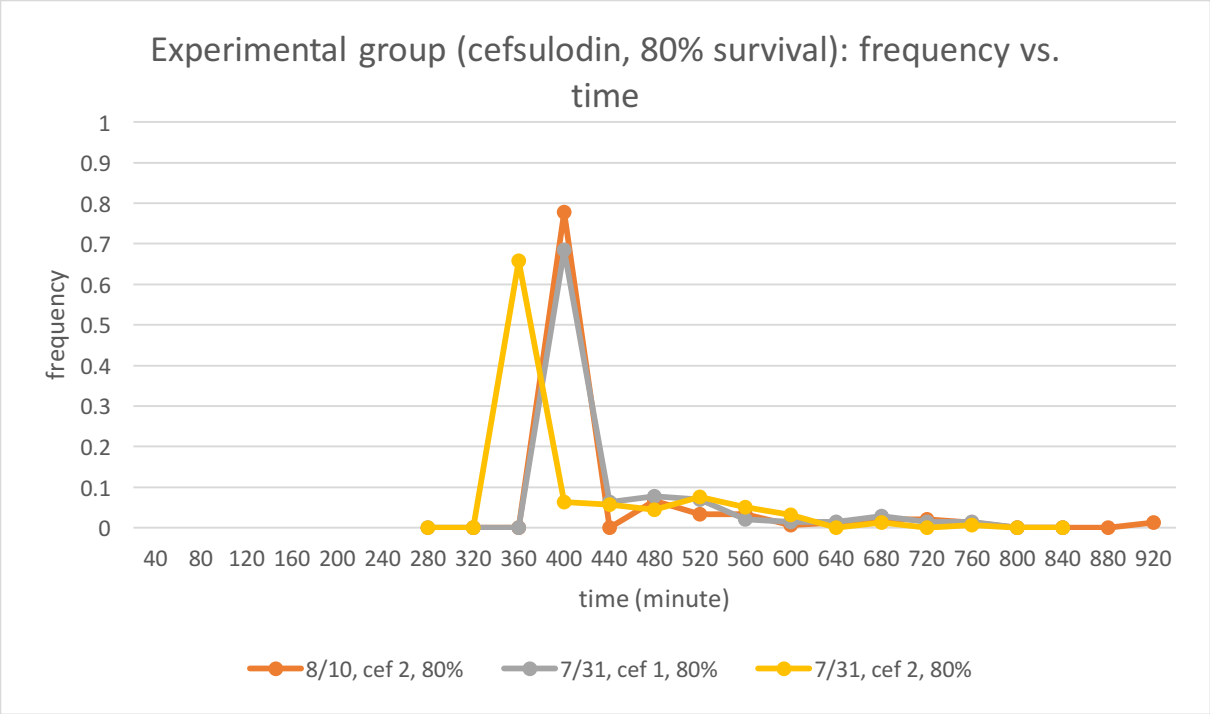

Figure 5. On experimental plates with cefsuodin that yielded 80% survival rate, colonies appeared later than those on control plates. The spread of appearance was almost twice the control plates.

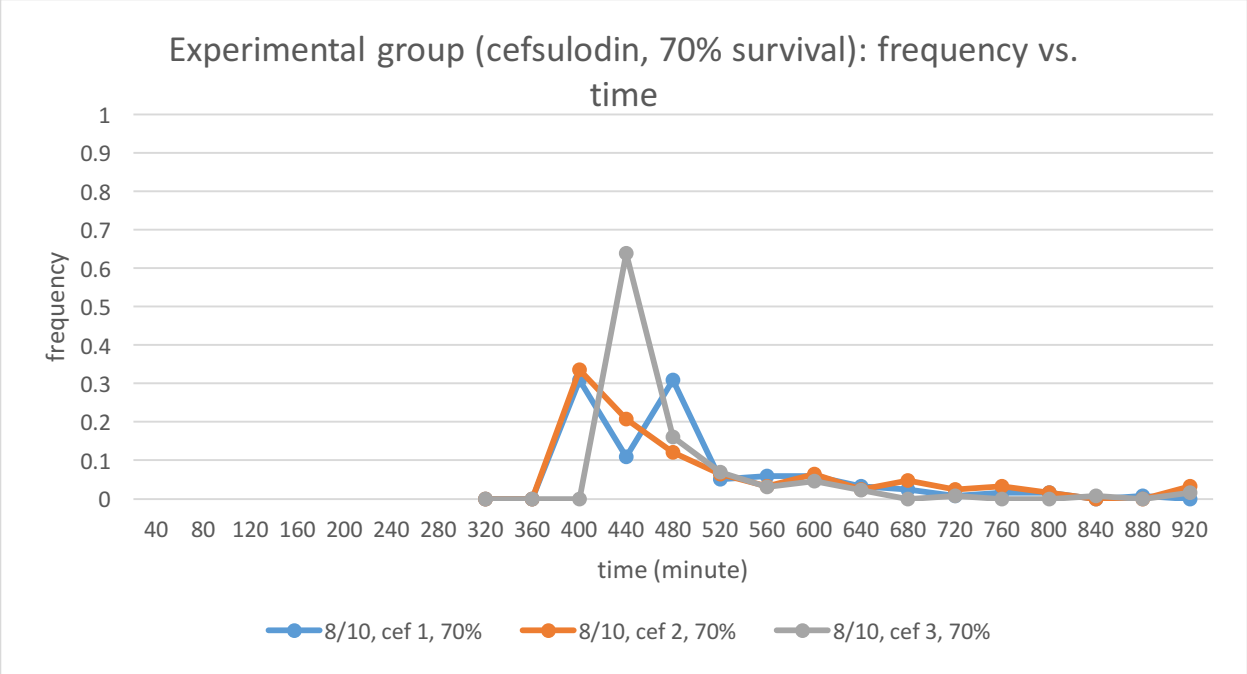

Figure 6. Comparing this to Figure 4 of control groups, only 10% decrease in survival rate resulted not only in significant lowering of peaks, but also a wider spread of colony appearance.

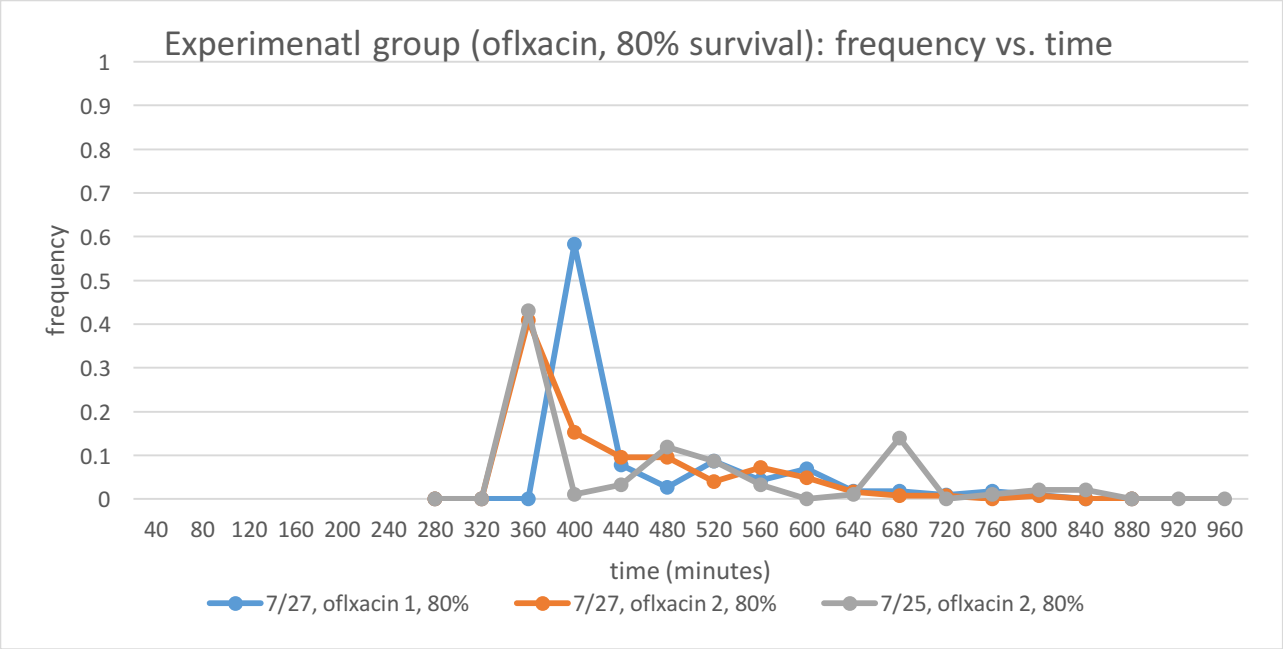

Figure 7. The delay in colony appearances and a wide spread were also present in plates with ofloxacin.

Based on abovementioned results, we drew a preliminary conclusion that when there is no antibiotic stress, growth of cells can be best described by the deterministic model. On the other hand, the wide spread of colony appearances in experimental plates with antibiotics displayed similarity with probability distribution curves of stochastic models (Figure 2). Therefore, the next step was to compare the stochastic model with experimental data in a more quantitative way.

Setting the parameter n (number of cells in each colony) in equation (1) to 10^4 and inputting designated birth and death rates returned a model that fits the experimental data quite closely. As shown in Figure 8, 9, and 10, the alignment of peaks and the spread of appearance of model and data agree with each other.

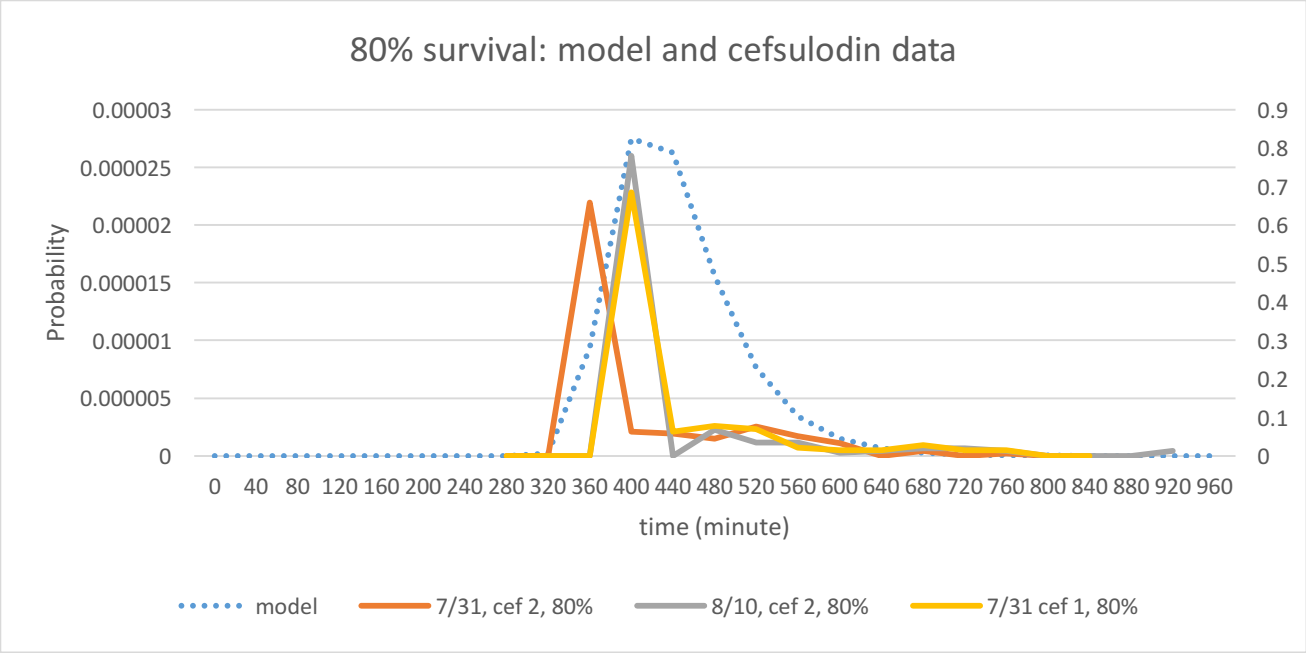

Figure 8. Fitting stochastic model to data from cefsulodin plates with 80% survival. The parameter n equals 10^4; birth and death rates are 0.027 (doubling/minute) and 0.0054 (doubling/minute).

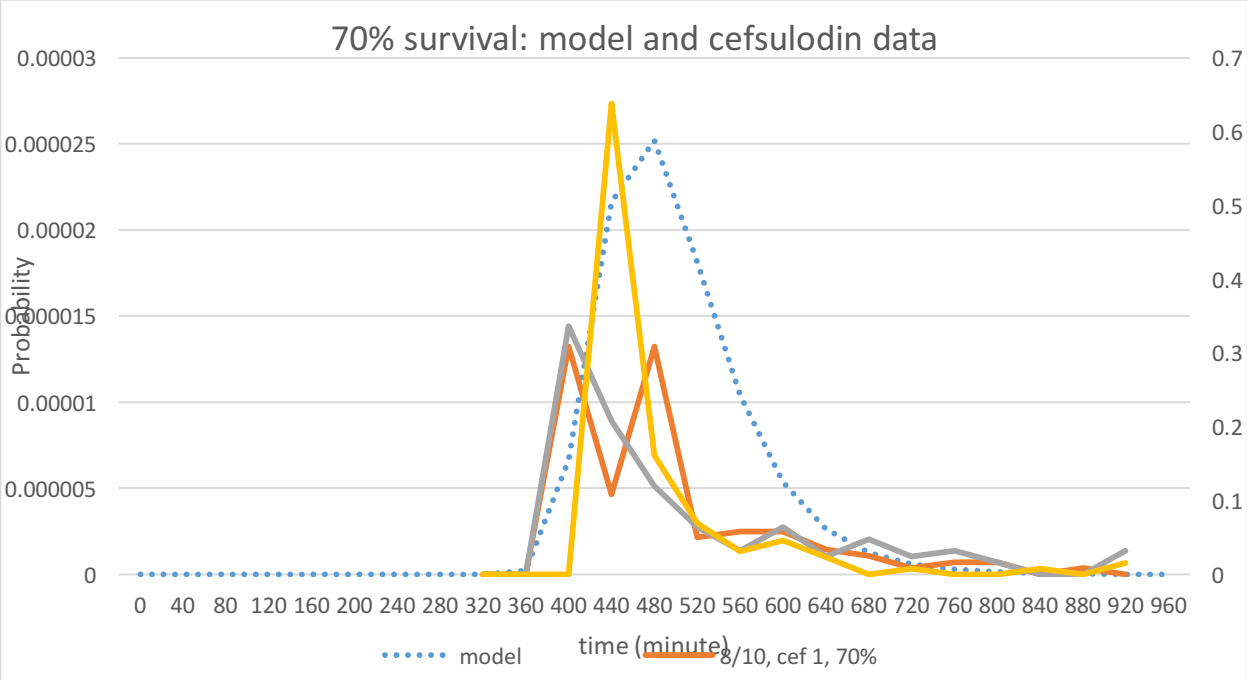

Figure 9. Fitting stochastic model to data from cefsulodin plates with 70% survival. The parameter n equals 10^4; birth and death rates are 0.027 (doubling/minute) and 0.0081 (doubling/minute).

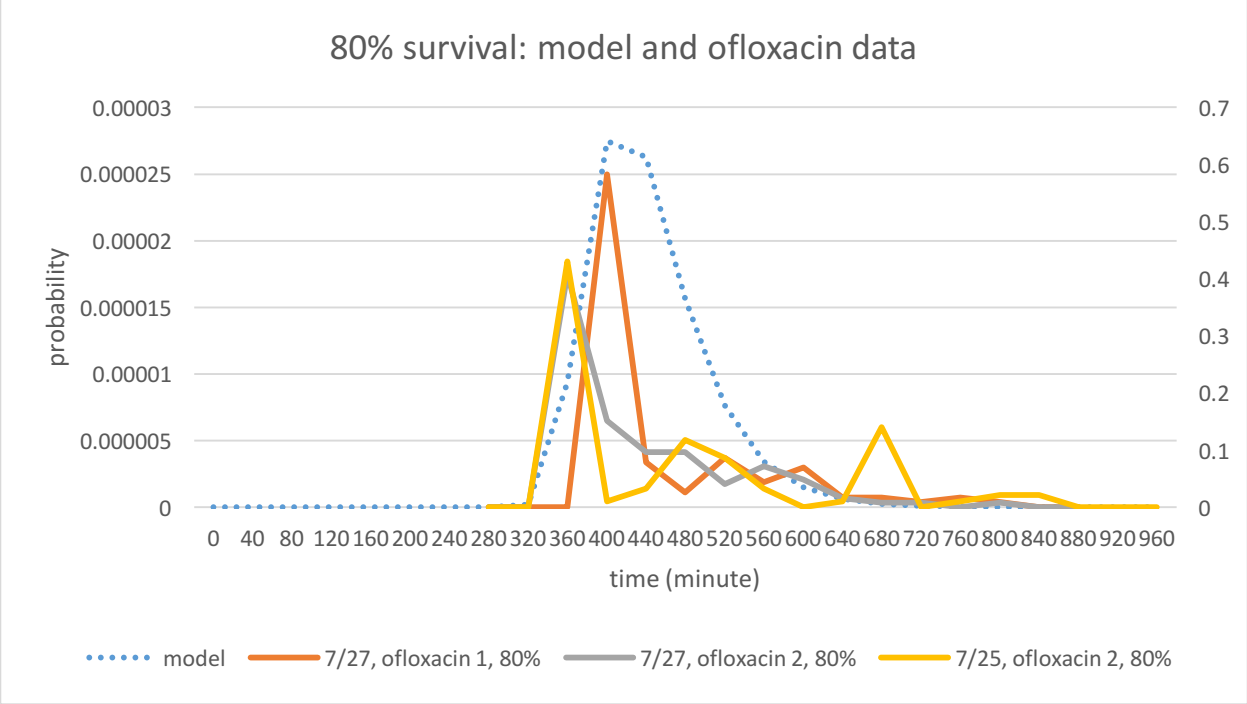

Figure 10. Fitting stochastic model to data from cefsulodin plates with 70% survival. The parameter n equals 10^4; birth and death rates are 0.027 (doubling/minute) and 0.0054 (doubling/minute).

Conclusion

After comparing colony appearance data to both deterministic and stochastic models of population growth, we concluded that when there is no antibiotic stress, the growth of bacteria follows the deterministic model. However, upon treatment with sun-lethal level of antibiotics, the growth aligns more with the stochastic model: although all colonies were growing on exactly the same medium and under same experimental conditions, they appeared at different time points due to the intrinsic stochasticity of this growth process.

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