iGEM 2011 HKUST Modeling Report

October 5, 2011

1 Introduction

Construction of this mathematical model is driven mainly to achieve several goals, among which is to illustrate and understand the biological influence of varying indole concentrations upon a mixed bacterial population. In addition, we can take a step further, using the model, to estimate the optimal strength of a particular promoter to express the enzyme complex T4MO.

The basis of this model relies on some known phenomena. Essentially, wild type *E. coli* produces indole at a basal level during pre-stationary phase of its growth while this production will be significantly increased once it enters the stationary phase. In an environment with antibiotic stress, only fully antibiotic-resistant cells (i.e. containing antibiotic resistance genes) will continue to produce indole while its antibiotic-sensitive counterparts (assumed "wild type") cease production. According to Lee et. al. (2010)[1], the secreted indole, once received by the wild type cells, would act as a signal to promote expression of efflux pumps, pumping out the antibiotics to prevent cell death. This phenomena is akin to indole conferring partial antibiotic resistance upon wild type bacterial cells.

It should be note worthy that a certain threshold level of indole keeps the wild type cells alive. (Experimental data by our group has shown that excess indole is highly toxic to the cells, which in turn, causes massive cell death within the bacterial population). Therefore, if we were to add more antibiotics or decrease the amount of indole within the culture, theoretically, we should be able to cause a significant decrease of viable cells. However, in a stable environment, addition of more antibiotics is futile or not practical (may exceed toxic dosage for medical purposes etc). Hence, decreasing the indole concentration appears to be a more viable approach.

Therefore, to reduce indole concentrations, we have employed the usage of an intracellular, mutant T4MO enzyme complex (toluene-4-monooxygenase from *Pseudomonas mendocina KR1* with mutations at G103L:A107G). Briefly, the environmental indole produced by the antibiotic-resistant bacteria will diffuse into the engineered T4MO mutant (*E. coli* containing the mutant T4MO gene), whereby the enzyme complex will convert indole into a variety of indole derivatives, in which 7-hydroxyindole is the major component, in short, degrading indole.

[1] Lee, H. H., Molla, M. N., Cantor, C. R., and Collins, J. J.(2010). Bacterial charity work leads to population-wide resistance. *Nature*, Vol. 467, p. 82-85.

Thus, we model the amount of indole in the culture, while taking into consideration the replication of the target wild type cells. Since there should exist a critical concentration when the target wild types become partially antibiotic-resistant, we then keep the indole concentration in the culture below that point, effectively removing any conferred resistance. In addition, we can also work out how strong the promoter should be in the expression of the T4MO enzyme complex in the ideal case.

2 Assumptions and Justifications

1. Assume that all the bacteria are of similar amount and cell volume. Therefore, we can simply add the concentration of three types of bacteria up to have the total concentration of bacteria in the culture.

2. Assume that the survival time of the indole-degrading bacteria with the mutated T4MO gene is much longer than its doubling time. Hence we can reasonably ignore the possibility that the bacteria will die before the end of its replication cycle caused by excessive accumulation of insoluble protein aggregates.

3. Assume that the natural degradation of indole is relatively slow with respect to that by the indole-degrading bacteria with the mutated T4MO gene.

4. Assume that the diffusion of the molecules (across the bacterial membrane) and bacteria (within the culture broth) are even and fast so that we can reasonably ignore the delay of degradation due to time consumed by diffusion.

5. Assume that there is no existing indole in the culture when the experiment is started, i.e. all the indole is made fresh by the bacteria.

6. Assume that the indole degradation rate of the T4MO enzyme complex (hence, T4MO mutant bacteria) remains constant throughout the experiment, while the indole production rate of each type of bacteria remains constant in the periods before and after the start of stationary phase respectively.

7. Assume that the amount of indole production and degradation per unit time is positively related to the amount of bacteria in the culture.

8. Assume that the mechanism behind the death of wild type bacteria is that its amount in the culture drops slowly and gradually as the comprehensive indole production rate is decreasing before the culture reaches a critical ratio of indole and bacteria concentration whereafter its amount drops significantly after the critical ratio is reached and exceeded.

3 Variables

I	the indole concentration in the culture
B	the bacterial concentration in the culture
t	the time before the experimental end point (or desired time before defined end point)
c	the critical ratio of indole and bacterial concentration in the culture that confers partial antibiotics resistance to wild type bacteria

For the bacteria with antibiotic resistance gene (suffix 1), the indole-degrading bacteria with the mutated T4MO gene (suffix 2), and the wild type bacteria (suffix 3),

v	the rate of the T4MO mutant degrading indole per unit concentration
v_{*0}	the production rate of indole before the start of stationary phase per unit concentration of the bacteria
v_{*1}	the production rate of indole after the start of stationary phase per unit concentration of the bacteria
$*_0$	the initial concentration of the bacteria (N for type 1, M for type 2, and K for type 3) where only the ratio of $N : M : K$ is needed
t_{*0}	the (mean) lag phase period of the particular type of bacteria
t_{*1}	the (mean) lag and exponential phase period of the particular type of bacteria
λ_*	the bacteria growth rate constant

4 Establishment of Model

Bacterial growth has four different phases: lag phase, exponential phase, stationary phase, and death phase. During the lag phase, the bacteria attempt to adapt to the new culture environment and has yet started to divide, while in the exponential phase, the number of new bacteria appearing (due to cellular division) per unit time is proportional to the present population. Thus, we have

$$\frac{dN}{dt} = \lambda N, \tag{1}$$

$$N = N_0 \cdot e^{\lambda t}, \tag{2}$$

where N is the total number of bacteria at time t, N_0 is the total number of bacteria at time $t = 0$, λ is the bacterial growth rate constant, and t is the time variable.

As bacteria (in this case, *E. coli*) undergo binary fission, we can rewrite the equation as:

$$N = N_0 \cdot 2^{\lambda t} \tag{3}$$

Since the bacterial growth rate constant λ is inversely proportional to the time of bacterial fission, the bacterial proliferation time obeys the exponential distribution,

$$T \sim \lambda e^{-\lambda t}, \tag{4}$$

where T is the bacterial proliferation time. Consequently, we have the expected bacterial proliferation time,

$$E(T) = \frac{1}{\lambda}, \tag{5}$$

which can also be interpreted as the mean time for each bacterium in the population to complete fission once. As the bacteria in the same population still possess non-synchronization, $E(T)$ also represents the mean doubling time of a bacterial population, i.e.

$$T_D = \frac{1}{\lambda}, \tag{6}$$

As for the generation time, denoted as T_G, stands for the time for a bacterium to complete the binary fission once under a standard condition. For bacteria undergoing synchronized fission, the generation time is exactly the same as the doubling time. Thus, we have

$$2N_0 = N_0 e^{\lambda T_G}, \tag{7}$$

$$T_G = \frac{ln2}{\lambda}, \tag{8}$$

and by comparing equations (6) and (8), we get the relationship between the generation time and doubling time:

$$T_G = ln2 \cdot T_D. \tag{9}$$

For the cases of non-synchronous fission, the expected or mean doubling time also has the above relationship with the generation time, which is crucial for transforming the generation or doubling time data into the bacterial growth rate constant λ.

Denote v as the rate of indole degradation by the T4MO mutant per unit concentration. Denote v_{10} and v_{11} as the indole production rate by the bacteria with antibiotic resistance gene before and after the start of stationary phase per unit concentration respectively, N_0 as its initial concentration in the culture, t_{10} as its (mean) lag phase period, t_{11} as its (mean) lag and exponential phase period, and λ_1 as its growth rate constant. Similarly, we define M_0 and K_0, v_{20} and v_{30}, v_{21} and v_{31}, t_{20} and t_{30}, t_{21} and t_{31}, λ_2 and λ_3 for the parameters of the indole-degrading bacteria containing the mutated T4MO gene and the wild type bacteria respectively.

To model the amount of indole in the culture I until the earliest start of bacterial death phase, assuming that $t_{30} < t_{20} \approx t_{10}$ and $t_{31} < t_{21} \approx t_{11}$, which is probably closest to the condition of our experiment, we have

$$\Delta I = \begin{cases} v_{10}\Delta t N_0 + (v_{20} - v)\Delta t M_0 + v_{30}\Delta t K_0 & \text{if } t < t_{30}, \\ v_{10}\Delta t N_0 + (v_{20} - v)\Delta t M_0 + v_{30}\Delta t K_0 2^{\lambda_3(t-t_{30})} & \text{if } t_{30} \le t < t_{20}, \\ v_{10}\Delta t N_0 2^{\lambda_1(t-t_{10})} + (v_{20} - v)\Delta t M_0 2^{\lambda_2(t-t_{20})} + v_{30}\Delta t K_0 2^{\lambda_3(t-t_{30})} & \text{if } t_{20} \le t < t_{31}, \\ v_{10}\Delta t N_0 2^{\lambda_1(t-t_{10})} + (v_{20} - v)\Delta t M_0 2^{\lambda_2(t-t_{20})} + v_{31}\Delta t K_0 2^{\lambda_3(t_{31}-t_{30})} & \text{if } t_{31} \le t < t_{21}, \\ v_{11}\Delta t N_0 2^{\lambda_1(t_{11}-t_{10})} + (v_{21} - v)\Delta t M_0 2^{\lambda_2(t_{21}-t_{20})} + v_{31}\Delta t K_0 2^{\lambda_3(t_{31}-t_{30})} & \text{if } t \ge t_{21}. \end{cases} \tag{10}$$

Then, we have

$$\frac{dI}{dt} = \begin{cases} V_1 = v_{10}N_0 + (v_{20} - v)M_0 + v_{30}K_0 & \text{if } t < t_{30}, \\ V_2 = v_{10}N_0 + (v_{20} - v)M_0 + v_{30}K_0 2^{\lambda_3(t-t_{30})} & \text{if } t_{30} \le t < t_{20}, \\ V_3 = v_{10}N_0 2^{\lambda_1(t-t_{10})} + (v_{20} - v)M_0 2^{\lambda_2(t-t_{20})} + v_{30}K_0 2^{\lambda_3(t-t_{30})} & \text{if } t_{20} \le t < t_{31}, \\ V_4 = v_{10}N_0 2^{\lambda_1(t-t_{10})} + (v_{20} - v)M_0 2^{\lambda_2(t-t_{20})} + v_{31}K_0 2^{\lambda_3(t_{31}-t_{30})} & \text{if } t_{31} \le t < t_{21}, \\ V_5 = v_{11}N_0 2^{\lambda_1(t_{11}-t_{10})} + (v_{21} - v)M_0 2^{\lambda_2(t_{21}-t_{20})} + v_{31}K_0 2^{\lambda_3(t_{31}-t_{30})} & \text{if } t \ge t_{21}. \end{cases} \tag{11}$$

Therefore,

$$I = \begin{cases} \int_0^t V_1 & \text{if } t < t_{30}, \\ \int_{t_0}^{t_{30}} V_1 + \int_{t_{30}}^{t} V_2 & \text{if } t_{30} \le t < t_{20}, \\ \int_{t_0}^{t_{30}} V_1 + \int_{t_{30}}^{t_{20}} V_2 + \int_{t_{20}}^{t} V_3 & \text{if } t_{20} \le t < t_{31}, \\ \int_{t_0}^{t_{30}} V_1 + \int_{t_{30}}^{t_{20}} V_2 + \int_{t_{20}}^{t_{31}} V_3 + \int_{t_{31}}^{t} V_4 & \text{if } t_{31} \le t < t_{21}, \\ \int_{t_0}^{t_{30}} V_1 + \int_{t_{30}}^{t_{20}} V_2 + \int_{t_{20}}^{t_{31}} V_3 + \int_{t_{31}}^{t_{21}} V_4 + \int_{t_{21}}^{t} V_5 & \text{if } t \ge t_{21}. \end{cases} \tag{12}$$

We can also easily get the bacterial concentration in the culture B:

$$B = \begin{cases} N_0 + M_0 + K_0 & \text{if } t < t_{30}, \\ N_0 + M_0 + K_0 2^{\lambda_3(t-t_{30})} & \text{if } t_{30} \le t < t_{20}, \\ N_0 2^{\lambda_1(t-t_{10})} + M_0 2^{\lambda_2(t-t_{20})} + K_0 2^{\lambda_3(t-t_{30})} & \text{if } t_{20} \le t < t_{31}, \\ N_0 2^{\lambda_1(t-t_{10})} + M_0 2^{\lambda_2(t-t_{20})} + K_0 2^{\lambda_3(t_{31}-t_{30})} & \text{if } t_{31} \le t < t_{21}, \\ N_0 2^{\lambda_1(t_{11}-t_{10})} + M_0 2^{\lambda_2(t_{21}-t_{20})} + K_0 2^{\lambda_3(t_{31}-t_{30})} & \text{if } t \ge t_{21}. \end{cases} \tag{13}$$

Other cases of t_{10}, t_{20}, t_{30} and t_{11}, t_{21}, t_{31} can also be computed accordingly.

Since the death of bacteria occurs throughout the process, which is also our ultimate goal, we only evaluate the process till the start of the death phase. Therefore, we note that t is less than the time till the earliest bacterial death phase.

Let c denote the critical ratio of indole and bacterial concentration in the culture that makes the wild type bacteria antibiotics-resistant. Therefore, we solve the equation for v_2

$$c \ge \frac{I}{B} \tag{14}$$

for all the cases after our desired time t to get the ideal dynamics of v_2.

After that, we can use statistical methods, for instance, moment estimation or maximum likelihood estimation, to fit the v_2 curve to the indole degradation rate equation.

For example, based on Le Chatelier's Principle, we may surmise that the dynamics of indole degradation rate seems most similar to the graph of the following equation and set it as the desired indole degradation rate equation:

$$v = alog(bt + 1), \tag{15}$$

where t is the time variable; $a > 0$ and $b > 0$ are both coefficients of the rate equation. Note that its graph should go through point (0,0).

Finally, we may experimentally relate the strength of promoter expressing the T4MO gene with the coefficients of indole degradation rate equation, which should be specifically studied, and find out the ideal strength of the promoter to express the gene of interest, T4MO.

For other cases using different bacteria (strains and/or species) and gene of interest, the model can also be applied with only slight modifications.

5 Strengths and Weaknesses

Our Model examines the relationship between the time and the concentration of indole in the culture. Using this as the foundation, we are able to illustrate the dynamics of a mixed bacterial population with respect to indole concentration changes. In addition, we can also find the optimal velocity of indole degradation when the concentration of indole in the culture fits our needs (e.g. exceeds the minimal concentration conferring partial antibiotics resistance etc). Such speculation is essential if we are to apply in cases such as understanding biological dynamics or for real-life applications (e.g. medical and industrial) etc.

However, the real values of the parameters needed, though theoretically accessible, still constitutes quite a big obstacle in the applications of the model. Some of the parameters may be extremely hard to accurately obtain. Also note that the relationship of the strength of promoter expressing the T4MO gene with the coefficients of indole degradation rate equation is yet to be rigorously studied theoretically or experimentally.

6 Further Improvement of the Model

As we have yet dig deeply into the mechanism behind the death of the wild type bacteria, the last assumption would appear inappropriate or less ideal. For a better model, we introduce a new variable, i.e. the survival rate of the wild type bacteria at different indole concentration S and define the perfection of our ultimate project objective as the $S \leq p$ ($p \in R$). Furthermore, we assume the relationship between the survival rate and the indole-bacterial concentration in the culture as $S = f(I/B)$.

For the $\frac{dI}{dt}$ computed in the original model in formula (11), we may multiply the real-time survival rate at the indole concentration S to the concentration of wild type bacteria in the culture. Therefore, the indole concentration at time 1 influences the survival rate and the indole concentration at time 2 which in turn affects the survival rate, forming a causal-effect relationship cycle. We may then program using the idea of the approximate computation of integral, Simpson's rule for instance, or other methodologies, to multiply $\frac{dI}{dt}$ with a relatively short time, say $\Delta t = 0.001s$ and

integrate it gradually, i.e.Δt by Δt, until the value of S finally reaches p. Therefore, we obtain the total time t, i.e. the sum of allΔts, which is exactly the expected time to achieve our ultimate goal. If we are to optimize the desired velocity of indole degradation and thus the optimal strength of enzyme expression, we may consider using the dichotomization technique to find the most efficient velocity based on our desired time. Therefore, if our project is to be applied in real industry (in contrast of studying the biological relationship of indole towards conferring antibiotic resistance), the ability of controlling time and the strength of enzyme expression is most appreciated for arranging the optimal industrial procedure.

7 References

Lee, H. H., Molla, M. N., Cantor, C. R., and Collins, J. J.(2010). Bacterial charity work leads to population-wide resistance. *Nature*, Vol. 467, p. 82-85.