BIOREPS Problem Set #14
Are bacteria more efficient than us?

1 Background

By now, you may have been persuaded that bacteria are evolutionarily superior to us humans. This may or may not be true. Of course, it depends on how you approach the question. One perspective, perhaps, may be to compare how well bacteria adapt metabolically to the availability of food in its present environment. In the 1940s, Monod observed that *Escherichia coli* growth rates increased with the increasing glucose concentrations. Monod's observation led to the development of one of the most widely used models in describing bacterial growth. However, Monod's growth model, or any growth model for that matter, does not provide insight into underlying evolutionary driving forces.[2] Fitness landscapes are mathematical surfaces that represent the organisms fitness depending on some biological property that can change due to evolution over time. These landscapes express the evolutionary principles for a given organism. Regardless of their supposed superiority, bacteria cells are a good place to study these fitness landscapes. In a recent *PNAS* paper, Maitra and Dill studied E.coli with the aim to determine whether E.coli bacteria maximize their duplication speed or their energy efficiency, or something completely different.[1] Ultimately, we want to relate the growth models and extensive growth data to fitness landscapes so that we may obtain what evolutionary principles drive bacteria cultures. Perhaps, it may even shed light on our question of superiority.

Upon studying various growth data, Monod described that bacterial growth has four distinct phases: lag phase, exponential growth phase, strationary phase, and death phase. Each phase is marked with a certain relationship between available nutrients and growth. The lag phase is observed when bacteria are placed in a new environment that supplies different nutrients than their previous environment. In this phase, the bacteria is adjusting to the new environment by expressing the necessary metabolic proteins. Once the bacteria has adjusted, it enters the exponential growth phase where the bacteria digests nutrients and seemingly dedicates the energy influx to duplication. At a certain point, the growth slows down and eventually reaches the stationary phase where the bacteria population becomes stagnant. The bacteria somehow recognizes that the availability of nutrients can no longer support such fast duplication rates. In this phase, the rate of growth is equal to the rate of death, where previously it was negligible. Finally, the depletion of nutrients causes death to predominate over growth leading to the gradual decrease in population.

In this problem set, we explore how bateria manage their energy influx to the production of ribosomal (RPs) and nonribosomal (NRPs) proteins. During the exponential growth phase, bacteria duplicate all their proteins quickly which requires proportionately more ribosomal proteins relative to the nonribosomal proteins. But the high costs of RPs are prohibitive when food is not abundant, so the bacteria switches gears to the production of NRPs which may support better management of energy. We will develop a mathematical model that will elucidate the evolutionary choices made by bacteria that we commonly observe as growth laws.

References

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2 Questions

2.1 Modeling *E. coli*'s Balance of Energy Flux and Protein Synthesis

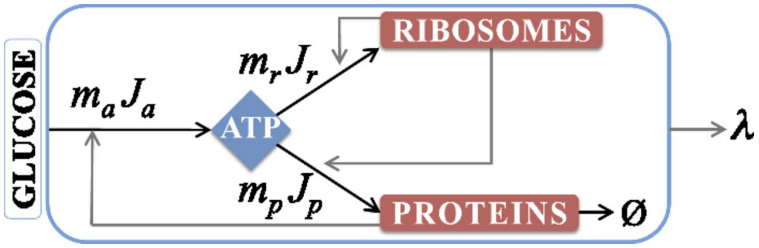

Figure 1: Compartment model of *E. coli*, where λ represents the specific growth rate of *E. coli*, and $\rightarrow \emptyset$ is the degradation of non-ribosomal proteins (the rate of which is γ). [1]

During the exponential phase of bacterial growth, the cells begin to grow and duplicate their proteins as fast as they can within the limitations of necessary materials. In particular, the cells create more ribosomes than other proteins during this process. A simplification of an *E. coli* cell's rates of synthesis and degradation during this exponential phase is shown in Figure 1.

a) To begin the process of modeling an *E. coli* cell, a set of differential equations describing the changes in concentrations of ATP, ribosomes, and non-ribosomal proteins (NRPs) must first be developed.

i) Write a set of equations $\frac{dR}{dt}$, $\frac{dP}{dt}$, and $\frac{dA}{dt}$ using only the rates within the box of figure 1 (so excluding the specific growth rate of *E. coli*, λ).

ii) Because this is the exponential phase, the *E. coli* cell is growing while the concentrations of ATP, ribosomes, and non-ribosomal proteins change. This in turn affects the concentrations of these molecules within the cell. How will the concentrations change as the cell grows? Starting with the equations from part i), factor the cell growth rate, λ, into each of the equations.

iii) During the exponential phase, over 80% of ATP in the cell is used to create proteins and ribosomes. Therefore, m_r and m_p, the stoichiometric numbers of ATP molecules needed to make ribosomes and NRPs, respectively, are 1 for $\frac{dR}{dt}$ and $\frac{dP}{dt}$. Set m_r and m_p equal to 1 for both of these equations.

iv) Using M_r, the molecular weight of RPs per ribosome, and M_p, the molecular weight of an NRP, write an equation describing the total protein density of the cell, ρ.

b) Recall that the flux of something can be represented as the product of its rate coefficient, its concentration, and a function that represents how the concentration changes, so if we are looking at the concentration of chemical X, $J = kf(x)X$.

Let k_r and k_p be the rate coefficients of ribosomes and NRPs, respectively, and let $f_r[A(G)]$ and $f_p[A(G)]$ be the functions for ribosomes and NRPs, respectively. Write the flux equations for ribosomes and NRPs, J_r and J_p.

c) Find the steady state equations of the 3 differential equations from part a). Find an equation for λ in terms of J_r and plug in the flux equations from part b).

2.2 Deriving Monod's Growth Law

Monod's growth law is similar to the Michaelis-Menten equation, but there is a key difference: Monod's growth law is empirical while the Michaelis-Menten equation is theoretical. The Monod equation is $\mu = \mu_{max}\frac{S}{K_s+S}$ where μ is the growth rate, $\mu_m ax$ is the maximum of said grwoth rate, S is the concentration of the limiting factor, and K_s is the value of S when $\frac{\mu}{\mu_{max}} = 0.5$.

a) From part $2.1c$, we have the steady state equation created by $\frac{dA}{dt}$ in terms of m_r, m_p, m_a, k_r, m_p, m_a, f_r, f_p, R, and P. Rewrite this equation in terms of m_r, m_p, m_a, m_p, m_a, f_p, λ, and γ. (*Hint:* look at the other state state equations–what does $\frac{R}{P}$ equal?)

b) In part $2.2a$, you should have gotten a quadratic equation in terms of λ (i.e. a$\lambda^2 + b\lambda + c = 0$). To simplify the result, you can write $\lambda_p = \frac{m_p k_p}{m_r}$ and $\lambda_a = \frac{m_a k_a}{m_p}$. Write out the quadratic equation in terms of λ, λ_a, λ_p, γ, and f_p.

c) We can also write λ_a in terms of λ. λ_a is a function of k_a, and $k_a = k_a^\infty f_g(G) f_a(A)$, $f_g(G) = \frac{G^{1.5}}{G^{1.5}+D_g^{1.5}}$, and $f_a(A) = \frac{D_a}{D_a+A}$. To rewrite A in terms of λ, we can rearrange $f_r(A) = f_r^\infty(1 - \frac{D_r}{A})$ solving for A using the steady state equation created by the differential of $\frac{dR}{dt}$ in both the average case, f_r, and the ATP saturated case f_r^∞. First, write A in terms of λ, then write λ_a in terms of λ.

d) By now, we have a quadratic function in terms of λ, λ_a, λ_p, γ, and f_p, and we have λ_a in terms of λ, so we can write the quadratic in terms of λ, λ_p, γ, and f_p. This will result in a cubic function of λ, where the coefficients, in no particular order, are -1, $\delta\lambda^\infty - \lambda_p f_p^\infty - \gamma$,

$\lambda_p f_p^\infty[\lambda_a^\infty(\frac{G^{1.5}}{G^{1.5}+D_g^{1.5}}) + \delta\lambda^\infty - \gamma] + \delta\gamma\lambda^\infty$, and $\lambda^\infty\lambda_p f_p^\infty[\delta\gamma - \lambda_a^\infty(\frac{G^{1.5}}{G^{1.5}+D_g^{1.5}})]$. In these definitions, $\delta = 1 + \frac{D_r}{D_a}$

e) The cubic function of $2.2d$ generates 3 real roots for γ, but only one is the observed glucose dependence of the specific growth rate, $\lambda(G)$, following the Monod Law. Varying G from 10^-3 to 1, set $\lambda^\infty = 1$, $f_p = 0.7$, $D_r = 0.18$, $D_a = 4$, $\gamma = 0.1$, $\lambda_a^\infty = \frac{m_a k_a^\infty}{m_p}$, $m_a = 30$, $m_p = 1950$, $k_a^\infty = 120$, $D_g = 0.07$, and $\lambda_p = 5$. In Matlab, use the function roots, and get a matrix of the 3 roots, each row refering to a set of roots. Plot the 3 roots againts the G values, and decide which set of roots–row 1, 2, or 3, is the correct set of roots. Why is this value of roots correct for λ, the growth rate of *E. coli*?

2.3 Cells Are Optimized for Energy Efficiency of the Fast-Growing Cells and Not Growth Rates of Efficiency Alone

Now we will try to determine under what conditions *E. coli*'s energy efficiency is optimized. We define its energy efficiency as the mass flux of all proteins produced divide by the molar flux of ATP synthesized, $\epsilon(\lambda, f_p) = \frac{\rho\lambda}{m_a J_a}$. We can then substitute the equation $m_a J_a = m_r J_r + m_p J_p$ to eventually get the equation $\epsilon(\lambda, f_p) = \frac{\lambda}{\Phi[\frac{\lambda}{\epsilon_r} + \frac{k_p' f_p}{\epsilon_p}]}$ where $\Phi = \frac{\lambda+\gamma}{\lambda+\gamma+k_p' f_p}$. The term in the square brackets is the total cost of synthesizing all proteins per unit time per ribosome, both RP and NRP. Further substitution can be done to eliminate $k_p' f_p$. $\epsilon = \frac{\frac{\lambda}{\lambda+\gamma}}{\frac{\lambda}{\lambda+\gamma}\frac{\Phi}{\epsilon_r} + \frac{1-\Phi}{\epsilon_p}}$. Finally we assume that some constants are fixed by physical limits. That is, there is nothing that evolution can do to further optimize these constants. These constants are protein synthesis k_p', degradation γ, ribosomal assembly k_r and relative costs of P vs. R, $\frac{\epsilon_p}{\epsilon_r}$.
a) Using the quations $\lambda^\infty = k_r f_r^\infty$ and $f_p^\infty + f_r^\infty = 1$ find an expresion for $\frac{d}{df_p^\infty}(\epsilon(\lambda^\infty, f_p^\infty))$ in terms of f_p^∞ and not λ^∞

b) Graph ϵ vs. f_p^∞ and find its maximum, where $\frac{d}{df_p^\infty}(\epsilon(\lambda^\infty, f_p^\infty)) = 0$. Use $k_p' = 10$, $\gamma = 0.1$, $k_r = 5$, $\epsilon_p = 18$, $\epsilon_r = 9$ as the values for constants.

2.4 The Cells Shifts Energy Flows Under Different Growth Conditions

In the previous part, we have found the cells are optimized for maximum energy efficiency, at fast growth. This means that the cells are evolutionary optimum when they create a balance between growth rate and energy efficiency by splitting the production of certain fraction of ribosomal and non-ribosomal poteins.

However, the cell is not always at its optimum state. As mentioned in the introduction, the cell growth cycle involves a total of 4 phases, and the growth condition is different for a different phase. In this part, we will see how this model corresponds to the changes in growth conditions. We will first start by plotting our model's variables against Glucose concentration

(G), using Monod law solution, obtained in 2.2. (Note: Even if you did not get 2.3, you will still be able to complete 2.4)

a) First, plot the graph of energy efficiency, ϵ against Glucose concentration, G (vary G from 0.01 M to 1 M).

Hint : Modify your code from 2.2 to produce only the correct solution that corresponds to Monod's law. Then use this solution for λ in the equations mentioned in 2.3, i.e. $\epsilon(\lambda, f_p) = \frac{\lambda}{\Phi[\frac{\lambda}{\epsilon_r} + \frac{k'_p f_p}{\epsilon_p}]}$ with $\Phi = \frac{\lambda+\gamma}{\lambda+\gamma+k'_p f_p}$. Use values γ=0.1, k'_p=10, f_p=0.7, ϵ_r=9, ϵ_p=18

b) The energy efficiency function can further be broken down into two components corresponding to individual efficiency contributions of R and P. The energy efficiency from (a) part then can be written as : $\epsilon = \epsilon'_r + \epsilon'_p = \epsilon_r j_r + \epsilon_p \frac{\lambda}{\lambda+\gamma} j_p$, where $j_r = j_r(\lambda) = \frac{\lambda}{\lambda+\lambda_p f_p}$ and $j_p(\lambda) = 1 - j_r(\lambda)$

On the same plot obtained from part (a), plot the individual efficiencies (ϵ'_r and ϵ'_p) against Glucose concentration (G)

Hint : Again, modify the code to substitute λ in each equation obtained from the solution of Monod's growth law from 2.2

c) Recall that ribosomal proteins carry out the growth of the cell, while NRPs catalyze the metabolism reactions including the biochemical conversion of glucose to ATP. RPs are used in replication, hence there chances of degradation are small, while NRPs need to degraded and formed at all times. The fraction of ATP flux used for NRP synthesis can be split between dilution and degradation. The fraction of degraded NRP can be given by : Degd = $(\gamma)(P)/(J_p)$ and the fraction of diluted NRP will be given by Dilt = 1 - Degd.

Plot the two fractions on a single plot against concentration of Glucose, G.

Hint: Use rate equation from 2.1 (b) and (c), and find the two fraction functions in terms of λ. Again, use Monod'law solution to plot the functions against G.

The plots obtained above can be interpreted as the following : Under slow growth conditions, small Glucose concentration (hence small λ), the cell is not efficient at converting energy to ribosomes or proteins. Most of the proteins being made by the cells are NRPs. But as it can be seen from plot of part (c), the NRPs degradation rate is very high under slow growth. Hence, most of the NRPs made are being degraded in the cell. thus, the cell is saving its energy by not making attempts to grow, but rather replenishing the NRPs. Under fast growth conditions, large Glucose

concentration, the cell has switched gears, and the energy efficiency of the cell is significantly greater. Part (c) plot also shows that under fast growth, the degradation fraction of the NRPs is significantly small, so less of the cell's energy is devoted to repairing degrading proteins. Now, the cell converts more sugar directly to cell growth.

d) It would make sense if the increasing sugar leads to an upshift of the production of ribosomes relative to NRPs, since, ribosomal proteins are the ones 'directly' responsible for the growth of the cell. Show that ϕ, mass fraction of all cellular proteins that are ribosomal, increases 'approximately' linearly with λ under fast growth. Hint : Use $\phi = \frac{M_r R}{M_r R + M_p P}$ to begin with and then use

justifiable approximations (including $k'_p f_p >> \lambda + \gamma$) at fast growth.