Evolutionary Dynamics
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Week 03
Lecture 15

Thank you. Hi everybody, let's continue our discussion of growth in batch cultures. As we discussed in the last lecture, the primary model setup for conducting growth experiments in bacteria and other single-cellular organisms is batch culture. So let's take a closer look at a particular growth curve. Again, our setup will be that we have a flask,

which has a certain volume, and we seed this flask with bacteria. This is shaking, and the temperature is 37 degrees Celsius. Presumably, there are nutrient conditions such that, as time progresses, the number of bacteria will increase. The number of resources available in the flask will decrease. That's intuitive.

We'll try to give this a little more mathematical sense in this video and the next one. So our goal today is to understand some of the kinetic features of this growth process. We want to see what happens as time progresses—how the number of bacteria in this flask changes. This is a growth curve. This is referred to as the growth curve of a microbial species.

And this characteristically has a few different phases associated with it. And let's just draw this, and then we'll see. So, as you can see, there are roughly four different phases this can be divided into. The first phase is where really no growth takes place. So, let's say this is still time t naught.

And the characteristic feature here is that there is no to very little growth. In the next phase, let us say up until this point, Let me redraw this a little bit more accurately. In this next phase, growth is happening exponentially. So, this is called the exponential phase of growth.

In the third phase, Let us say this happens till time T1. In the third phase, T1 to T2, the number of bacteria in the flask more or less stays constant. So, this is the phase when all the nutrients have been used up. So, the number of bacteria stays more or less constant.

And in the fourth phase, there is active death taking place. And as a result, the number of bacteria decreases with time. And if we let this go on for a sufficiently long amount of time, we will reach a stage where there are no viable bacteria left in the flask. Let's try to understand what is happening

in each one of these phases. So what we discussed in the last video was that the carbon source present here is lactose.

Lactose is a disaccharide of glucose and galactose. So we have, let's say, the circle represents glucose, and the square represents galactose. So when glucose and galactose are linked together, the resulting disaccharide, which consists of two sugar molecules, is referred to as lactose. And the way E. coli utilizes lactose for growth is, first, it has to internalize it. So let's say this is an E. coli cell.

First, it has to bring the lactose inside the cell. So this transport process has to take place. That's step number one. And the second step that has to take place is that this lactose has to be broken down into glucose and galactose. So the bond that links the two sugars has to be broken.

And now the cell has access to free glucose and free galactose, which will then be utilized by the cell for growth and energy purposes. E. coli and most species cannot use—well, no other species can use lactose as it is. It has to be broken down into glucose and galactose. And that's step number two: the breaking down of lactose into glucose and galactose is step number two. But these two steps are facilitated by specific proteins in E. coli.

And the first step is facilitated by a dedicated transporter called lacY. And this protein Its only job is to transport lactose from outside the cell to inside the cell. And this protein, as we saw in one of the previous lectures, is only produced when lactose is present in the environment. If you place E. coli in an environment where glucose is the only carbon source, then lacY will not be made.

The hydrolysis of lactose into glucose and galactose is facilitated by another protein called lacZ. which is also only synthesized when cells are in an environment where lactose is present as the carbon source. When we first seed this culture with this one bacterium, let's imagine that it comes from a condition where it was growing on glucose. and then we place it into a flask where the source of energy and carbon is lactose. What that means is that when I seeded this culture, there was no lacY or lacZ in this cell that I placed in the flask.

As a result, because it lacks the necessary proteins to grow on lactose, There is going to be no growth in the culture that I witness because the cells that I placed there are not ready to utilize lactose, as they don't have the necessary proteins for the breakdown and internalization of this disaccharide. This not being ready to utilize the resources that are present in the current environment leads to this phase of no growth, and this is referred to as the lag phase. That's the first phase of growth that characterizes bacterial growth in a batch culture, which is the lag phase. After a while, though, the cells, in practical terms, seeding will not happen with one cell.

There will be several cells that I seed with. And hence, after a while, these cells would have made a sufficient amount of lacY and lacZ to start exhibiting growth. And in this condition, where the initial density is low, so at this point, when cells have made lacY and lacZ, the number of cells in the culture is small, nutrients are plenty, and as a result, they start dividing exponentially. So two become four, four become eight, eight become 16, and so on and so forth. As a result, with time, this phase of growth exhibits this exponential increase in number.

Hence, for this reason, this phase is called either the exponential phase or the log phase of growth. And this is the second phase associated with bacterial growth. But soon what's going to happen is that because the number of bacteria in this flask is growing exponentially, and with that exponential growth, every individual cell is placing its demand on lactose and other resources that are available in the flask. Resources are going to become very limited. And as a result, this growth will start to decline.

And after a while, there is no more growth taking place. And as a result, the number of bacteria in the flask is more or less constant. And this is why this phase of growth is referred to as the stationary phase of growth. And in the stationary phase of growth, what happens here is that the number of births is exactly managed—is exactly balanced by the number of deaths taking place.

So what's happening in the stationary phase is not as if there were n number of individuals and the n stay the same throughout, but what's happening is that some bacteria die; when they die, they lyse, and the constituents of these bacteria are released into the extracellular media, and these constituents provide some resources to facilitate a minuscule amount of growth. So in this phase of growth, the births are facilitated by death and lysing of cells which are no longer alive. These two processes are balanced with each other, leading to the overall number of bacteria in the flask staying constant. And this is referred to as the stationary phase of growth. But this is not sustainable.

Eventually, the stress of not being able to grow and maintain homeostasis is going to be too much, and numbers are going to decrease in this death phase as we go forward in time. And depending on how deep we go here, how deep we go here, this is going to eventually go down to zero, where there'll be no viable bacteria left in the flask. So what we should... Focus here is that in these four phases of growth, let's say this is phase one, phase two, phase three, and phase four. Bacterial growth is characterized by its performance in each one of these four phases.

So we'll try to understand some numerical estimates of what allows us to define this growth process. So again, let's go back to our growth curve, and we get something like this. So in the first phase, This is the lag phase. The characteristic of the lag phase is the duration that the bacteria need to exhibit, to start exhibiting growth in the environmental condition that it's being placed into.

So this is characterized by its duration. So the relevant parameter here is that the lag phase, by definition, does not exhibit any growth. So what defines the lag phase is the amount of time it takes bacteria to break out of this phase where there is no growth and start dividing. So it is characterized by duration, and hence it could be measured in minutes or hours. Some unit of time.

What you should note at this point is that suppose I have a flask, and this flask contains lactose as the carbon source. In that case, when I seed this with one bacterium, if this one bacterium is coming from an environment where it was growing on lactose, in that case, Because in the previous flask from wherever it is coming, because it was growing on lactose, it already had lacY and lacZ already present in it. So these two were present in it, and hence when I transition it to another flask which also contains lactose as the carbon source, the lag is going to be very short. Because it was already growing in an environment, and I'm simply placing it in an environment with the same resources, only its quantity is different.

And hence, it doesn't need any time to make these proteins and then start utilizing lactose. It's already ready to use lactose and exhibit growth. On the other hand, if this individual came from an environment where it was growing on glucose, this individual does not have any lacY or lacZ, and hence it's going to need some time to make those proteins, and only then will it be able to exhibit growth. So in this case, the lag is going to be long.

This could be several hours before it starts to exhibit growth. But what we should take into account here is that this duration of lag is independent of the genotype. Their DNA is exactly the same. But the duration of the lag is different in the two cases because of the environment from which they are coming, not because of the genotype that they are carrying. The genotype of the two individuals is exactly the same.

So the lag phase duration is measured in time and is dependent on the prior culture conditions. That is not to say that it's completely independent of its genotype. It's obviously also dependent on its genotype. For instance, if these two genotypes were different from each other, and this purple individual carried a mutation such that lacZ is no longer working in it.

If lacZ is no longer working in it, that means this individual can never make lacZ, which means that hydrolysis of glucose and galactose into, hydrolysis of lactose into glucose and galactose cannot take place in this individual. As a result of which, this individual can never exhibit growth in an environment where lactose is the only carbon source. So and that results because of this mutation that it's carrying and hence its genotype. So duration of lag phase is dependent on prior culture condition and the genotype of the organism and is measured in amount of time it takes an individual genotype to start exhibiting growth. Let us move to the next phase.

In the exponential phase or the log phase, growth is exponential. And as we will see, let us say that the number of bacteria at the beginning of exponential phase is N0. then growth is taking place exponentially. And hence, in this phase, the number of bacteria at any given point in time, let's say NT, is simply equal to N naught into E to the power RT. What that means, let's write this a little more clearly.

In the exponential phase, NT is equal to N naught e to the power RT, where NT is the number of bacteria at time t. So this is in context with our growth curve. and we're talking of this particular phase. And this is the number of bacteria at the beginning of the exponential phase or the log phase. And let's say this, as far as the log phase is concerned, this time here is t equal to zero. And at any given point,

At time T in the log phase, if we were to estimate how many bacteria are here, that's NT. This number can be estimated using this formula. Where NT is the number of bacteria at time T since the start of the log phase, N0 is the number of bacteria at the beginning of the log phase. And we have one more variable here, which is R, the growth rate. Which means that once the bacteria start to divide in the log phase, how fast do they divide?

So for faster-growing variants, the faster the growth, the greater the R. Because as far as the log phase is concerned, we have one bacterium which divides into two. But then this divides again, and we have four, and so on and so forth. This is the definition of the log phase. However, what the log phase doesn't tell you is how long each one of these processes took.

T naught. If this is happening really rapidly—if growth is happening every 20 minutes—then that's a very high value of R. However, if it's taking three hours, then that's a smaller value of R. So in this log phase, growth can be estimated via this formula. In the next phase of growth, The first phase, lag, is characterized by its duration. Log is characterized by R, its growth rate.

Stationary phase. It is also going to be characterized by its duration. Which is to say that once the bacterial population has reached a certain density, once it has reached a certain density, let's say after t-dash amount of time, how long is it before numbers actually start falling? Let us say this is double-dash.

So, what is this duration of time delta t before numbers start decreasing? And what you should realize here is that in this stationary phase, the death process is actually exactly balanced by the birth process. However, beyond the stationary phase, the number of deaths is greater than the number of births. So what we are asking here is how long does the population carry its resilience such that the death and birth processes are closely matched with each other, beyond which the death

rate is going to exceed the rate at which births are taking place, and the overall number of bacteria are going to decrease with time.

The longer the stationary phase associated with a species, it is in some sense a representative of how resilient the species is to tackle the stress and not let the death rate exceed the birth rate associated with the process. So these are the three important phases of growth that we'll be focusing on. Let's now do this toy example where we imagine that we have these two species. We are going to represent time against the number of bacteria. So species one exhibits a growth curve like this.

If we start species one with N naught number of individuals, it exhibits a certain lag phase duration and then exponential growth. But then there is a stationary phase followed by death at a certain rate. So that's the characteristic feature of growth associated with species one. Let's compare this with species two, which is also seeded at the same number, N naught. And let's say that species two exhibits a slightly different growth characteristic.

And so on and so forth. This is species two. So now the question before us is: if individually grown in a flask, If these two species are individually grown in a flask, that's the characteristic growth feature they exhibit by themselves. But if I were to grow them collectively together, let's pose this question as follows: which of the two species

is fitter if grown together in a flask? And as we can see, the answer to this question is not as straightforward as it might appear. If we compare their characteristics in the growth phases, we have lag, log, stationary, and death phases. We have species 1 and species 2.

If I am looking at only the lag phase, then up until this point, there is no difference between the performance of these two species. However, if I am looking at the end of log phase of species 1, then I note that the log phase associated with species 1 is longer compared to the log phase associated with species 2. So by the time species 1 starts to grow, species 2 has already started growing for some time. And as a result, if the comparison that I am making for fitness is during the log phase, then 2 is fitter than species 1. So if the comparison is in log phase, species 2 is fitter.

And again, I'll repeat the reason because in this lag phase, species 1 is not growing at all, whereas species 2 starts to grow faster earlier as compared to species 1. So while species 1 numbers remain constant at a 0, species 2 numbers start to increase before species 1 enters its log phase. Secondly, now if we move to log phase, we note that species 2 grows at a rate which is much more rapid as compared to the growth rate which is exhibited by species 2. So 1 is growing faster as compared to 2, as a result of which, if my comparison of fitness now happens in a log phase, then species 1 is winning.

Next, we said that stationary phase is some sort of a measure of resilience of a species to keep the death rate balanced exactly by the birth rate. If we look at how long these stationary phases are for these two species, for species one, this is the duration of the stationary phase. Of course, because it was growing very rapidly, it entered stationary phase faster as compared to species 2. Whereas for species 2, the stationary phase is much longer as compared to that of stationary phase. So let us say this is delta T for species 1 and this is delta T for species 2.

Delta T2 is greater than delta T1. So, as far as the stationary phase is concerned, Species 2 is able to keep its number constant for a much longer time compared to species 1 because, after delta T1 time, species 1 numbers start to decrease. So, if we are comparing fitness in the stationary phase, then species 2 does better compared to species 1. And lastly, when they enter the death phase, we see that the fall in numbers for species 2 is extremely rapid.

So, while it maintains its stationary phase for a long time, its fall in numbers is extremely rapid, and it goes to zero in a very short amount of time. Compare that with species 1, where the numbers are decreasing much more gradually, and hence it's able to resist death much more compared to species 2. And as a result, in the death phase, species one is fitter compared to species two. So, the point of this exercise is that now we have these four measures of growth and fitness in a simple growth curve. And when we compare these two species, sometimes one will do better compared to the other.

In some performance criteria, whereas in another performance criteria, the other species will do better. So, we need, it's not clear looking at this table which is fitter. Because although if I do the growth curve till here, Although species one is fitter, in the preceding three regimes that were there, species two was doing better. So, what is going to be the sum total of the fitness effect of all of these four phases of growth that I'm looking at?

For this reason, we need to develop some quantitative understanding of how to compare growth curves and what would happen if I grew them together in a single flask. And that is what we'll continue with in the next video.