

Subject: Bacterial Growth

Lecture No: 6

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Bacterial Growth and Culturing

- **Bacterial Growth** by definition is the increase in the number of bacterial cells NOT the size of the bacterial cell. Opposite to humans, or trees where we use the term “growth” to refer to an increase in size or advance in time.
- For example, if we expose a bacterial cell to an antibiotic in order to study its action and come back to observe that the bacterial cell has only increased in size, we know that the antibiotic inhibited the bacterial growth. This is because, as long as there is no increase in number, we know that it has been inhibited.

- **Mechanisms of bacteria growth:** -

- 1) Binary Fission
- 2) Budding

- **Binary Fission:**

This is the major method of bacterial reproduction.

- 1) Usually a bacterium has one single unpaired chromosome.
- 2) The parent cell synthesizes a large amount of enzymes, protein, ATP, and other cellular components.
- 3) Then, the cell makes a copy of its chromosome.
- 4) Due to the absence of mitotic spindles in the bacterial cell, there are certain structures on the plasma membrane which chromosomes bind to. One chromosome binds at each pole.
- 5) Afterwards, a transverse septum grows in the middle of the cell – made up of peptidoglycan-, it continues to grow until we have two daughter cells. It either divides without complete separation which gives the bacteria its arrangement/shape: tetrads, sarcinae, streptococci **or** it undergoes a complete separation resulting with 2 separate daughter cells each containing a single chromosome copy.
- 6) The parent cell is now non-existent as it has become 2 daughter cells with identical genotype (genetic make-up/composition) and phenotype (morphological and functional properties of the cell that results from the expression of the genotype)

Note (1): All the cells in human body are composed of the same genotype i.e. genetic composition. However, they have different phenotype due to having certain genes expressed and others suppressed. (Example: nerve cell and muscle cell)

Note (2): Why we didn't call this process mitosis? Because even though it results with the same products of mitosis, yet the actual mechanism by which it occurs by is entirely different. (No spindle fibers, no chromosomal splitting, etc.)

Note (3): The bacterial reproduction is continuous as long as the resources are available, and the conditions are supportive for its growth. It stops only when the conditions become unfavorable.

Note (4): The Generation Time: The time needed for a bacteria cell to go through one cycle of binary fission. On average for most bacteria it is 30 to 60 mins. However, there are exceptions (including the mycobacteria) which take hours. In order to find the time needed for a single bacteria cell need to reproduce 20 generations we multiply the generation time by 20.

Each cell gives rise to 2 daughter cells. After one generation we have 2 cells, after two generations: 4 cells, after three generations: 8 cells ...etc. Accordingly, the growth is exponential which has much higher rate than a linear growth. The equation for the number of bacterial cells after a number (N) of generations is 2^N . Therefore, after 10 generations there are 2^{10} (1024) bacterial cells.

- Budding:

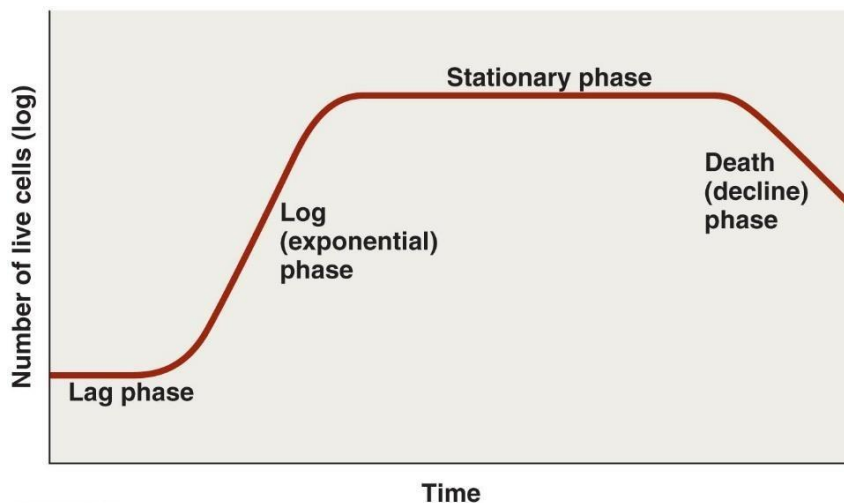
This is the major mechanism of reproduction of yeast (a unicellular fungi). Very few bacteria undergo budding as a method of reproduction.

- 1) Like the binary fission parent cell increases its components and enzymes.

- 2) The parent cell with a single unpaired chromosome makes a copy of its genetic material (chromosome)
- 3) It forms a vesicle similar to that in exocytosis. Then it inserts the chromosome copy inside the newly formed vesicle.
- 4) The vesicle is released
- 5) The products of this mechanism include a much smaller daughter cell, and the same parent cell still exists, which is much larger and more mature than the newly formed daughter cell.

Note (5): Although both are asexual forms of reproduction where two genetically identical cells 'clones' are produced; in binary fission the parent cell is divided into two equally sized new cells. Whereas, budding produces a small new cell in addition to the existing parent cell with different phenotype.

Standard Bacterial Growth Curve



In order to understand this curve, we will discuss bacterial growth in a liquid growth media.

- **Liquid growth media:** an aqueous solution containing nutrients and bacterial requirements for growth.

- Scenario One: If we incubate 10 cells in a liquid growth media with optimum conditions, and we monitored it over time, what will happen to the number of the bacterial cells? It will increase.
- **Lag phase**: Yet if the generation time is 30 minutes, and we only left it for 30 minutes and came back to observe, we will not notice any increase. This is due to the time needed for the bacteria to adjust and adapt to the new environmental conditions. The bacteria are very much efficient in adaptation nonetheless it needs time. This is why there is a certain period of time where the number of bacteria will not significantly increase. This is called the **Lag phase**.
- Lag phase may last from a couple of minutes up to several hours depending on:
 - A) The type of bacteria
 - B) Its ability to adapt to the new conditions
 - C) The previous and new conditions.

In other words, as the difference between the previous and preceding conditions increase the time needed for adaptation will increase.

Note (6): In the Lag phase – Cells don't increase in number, but are metabolically active, the cells are increasing in size, incorporating various molecules from the medium, synthesizing enzymes & and producing large quantities of ATP (energy).

- **Log phase (the most active in growth)**: After the adaptation of the bacteria, the cell replication/binary fission will start to take place; here, the number of bacterial cells increases exponentially. The curve above has the x-axis as a linear scale, however the y-axis is exponential/logarithmic scale (i.e. it would have numbers as 10^n , where n is 1,2,3,4...etc.) This is called the **log phase** because the bacteria will be increasing in logarithmic/exponential fashion. All the bacteria undergo an exponential increase (trend) in the log phase yet not all have the same rate/slope of growth.

We use chemostat to lengthen the log phase which has inlet of nutrients and outlet of waste.

- Rate of growth >>> Rate of death (negligible)

- **Stationary phase:** This phase doesn't last forever, because as we go on with the bacterial growth the nutrients will be depleted and competition on nutrients will take place and waste products will accumulate. Also, space limitation will be a factor contributing to this as well. Accordingly, the growth rate will decrease and the death rate will increase. Death rate=growth rate explains the plateau presented in the graph. This is called the **stationary phase**. When cell division decreases to a rate equal to the rate of cell death, the number of cells remains constant, which appears as horizontal straight line on the bacterial growth curve – In this stage, the medium contains limited amount of nutrients & may contain toxic quantities of waste materials. O₂ is limited to aerobic organisms & damaging pH changes may occur.
- **Decline phase:** When the death rate significantly increases and the number of bacterial cells decreases, the decline in the graph results. This is called the decline phase. Medium is less & less supportive to cell division, so cells lose their ability to divide and die. The number of living cells decreases by logarithmic rate (the probability of every cell to die is the same because every cell is exposed to the same conditions). – The duration of this phase is highly variable like the logarithmic phase. Both depend on genetic characteristics of the organism. Some bacteria contain few bacteria that remain alive after months or years.

When the bacteria are at their best state?

The bacteria are in their best state and best metabolic activity at the log phase. Therefore, if we try any treatment (antibiotic) against the bacteria, we extract bacteria from the log phase to keep the bacteria at low numbers until the immune response matures. Because if we extract it from the decline phase, it will not clearly indicate for the activity of the treatment; because bacterial cells are already dying.

When to harvest antibiotics?

Antibiotics are best harvested when the bacteria are in the stationary or decline phase. This is because when competition arises between different microorganisms over nutrients, oxygen and space; the cells produce antibiotics to kill each other.

What determines the duration for each of the following stages?

1) Lag phase:

- bacterial kind
- current conditions
- previous conditions
- bacteria's ability to adapt

2) Log phase, stationary phase and the decline phase:

- bacterial kind
- current conditions

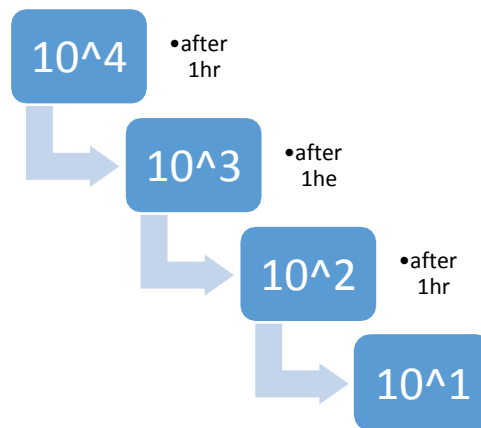
- **Solid growth media:** a solid media (an agar media –a gelling polysaccharide agent-) with suspended nutrients.
- Scenario Two: If we put a single bacterial cell on the surface of an agar plate, then incubated it. What we will notice on the next day? We'll see that the bacteria have created a colony: millions of cells at close proximity.
- Each single living bacterial cell will divide to form a colony i.e. each bacterial cell represents a colony forming unit (CFU).
- If we look at the bacteria in this colony, we will find that all phases of growth occur simultaneously in a colony; they are at different phases of growth, some will be in the lag phase while others will be at the log phase or decline phase. Opposed to the bacteria in a liquid growth media, where they all are at the same phase simultaneously, because the liquid is homogenous.
 - In solid growth media, if we observe the cells at the bottom- center, which have a space and oxygen limits, we find them at the stationary or decline phase. However, if we look at the cells at the periphery of the colony, these cells have fresh nutrients, vast space and a sufficient supply

of oxygen; they are most likely at the log phase growing rapidly. This is why it is hard to draw a standard bacterial growth phase for bacteria in a colony since it will not be clear nor representative.

- **Bacterial Death:**

“If we exposed a bacterium to conditions that will result in bacterial death, how the bacteria will decrease in number? Linearly or exponentially?”

For instance, if we expose bacteria to heat treatment (60 degrees Celsius), the number of cells will be as follows:



- 10000-1000 (9000) cells killed at the first hour, $9000/10000 * 100\% = 90\%$ percent death.
- This means that the probability for the bacteria to die is 0.9, and the percentage risk is 90%.
- Since all the bacteria are exposed to the same treatment, this means that the probability of the cell death is 0.9 for all bacteria cells. Thus, we can say that we have a death trend of 90% of the population every hour, regardless of the bacteria's original number. This trend of bacterial death is exponential.
- It is exactly the same for any other cause of cell death, not only heat treatments.

Note (7): Pasteurization is the heating of any product till 70 degrees Celsius, resulting in death of microorganisms and vegetative cells. However, we do not consider it sterile because 70 degrees will not destroy endospores.

Measuring Bacterial Growth – Enumeration of Bacteria

- In order to study the effect of a Bacteriostatic antibiotic, we are going to take a sample of 10^3 cells per ml (bacterial density) and expose it to this bacteriostatic antibiotic for 24hrs. If it increased → it is resistant. If the number stayed the same → we have inhibition, and the antibiotic is efficiently working.
- The problem is that we need to find a way to count the number of cells.

1) Direct Microscopic Count

- Take a small volume 0.1ml. Smear it on a glass slide and count using a microscope.
- This is used for measurement for a certain metabolite, yet it is not frequently used.

2) Colony Count Method spread plate method

- Take 0.1ml and spread it on an agar plate (9-11cm in diameter). The 0.1ml might have around 100 bacterial cells spread over the surface. If we are to incubate it and come back the next day we can count the number of colonies (visible) and accordingly know the number of bacterial cells in the 0.1ml. 4 colonies= 4cells (more accurately: 4 colony forming units).
- The assumption made here is that each colony originated only from a single cell.
- Now we say there are 4 colony forming units in the 0.1ml, which is 40 colony forming units in 1ml. This means that this is a **bactericidal** agent since it decreased from 10^3 to 40.
- Most important, reliable and commonly used method.

- Major advantage: differentiates between viable and dead cells. A dead cell will not form a colony. It gives a very good estimate.
- However, the problem is that the bacteria is usually present in a much higher number than this (>300). So we need to do something called the tenfold serial dilution. Supposedly we have a bacterial culture with 10^6 cells per 1ml. If we take 0.1ml from it, we will have 10^5 cells which is a huge amount; and we can't see discrete 10^5 individual colonies. On the contrary we will find continuous growth/serial suspension. Therefore, we undergo tenfold serial dilution $10^6 \rightarrow 10^5 \rightarrow 10^4 \dots$ etc to dilute to a number above 30 and below 300. Then transfer 0.1ml to agar plate. The transfer is done either by:
 - a. Pour plate method (will be explained next lecture) or
 - b. Spread plate method (The method explained above)
- This works for 30-300 cells. Above 30 to have a statically representable sample, and below 300 since anything above will not go with our assumption: every colony represents a single cell, as it will end up forming a continuous colony.

GOOD LUCK