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# Microbiology

Doctor 2017 | Medicine | JU

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**Done By**

Shehab

**Contributed in the Scientific Correction**

Yousef Omar

**Contributed in the Grammatical Correction**

Yousef Omar

**Doctor**

Anas

**Bacterial Growth and Survival**

After discussing the structure of a Bacteria, we must know how it survive and grow in a specific media.

Firstly, the survival of any microbial group within an environment depends on 2 factors: 1- The availability of **Nutrients** in the media. 2- The ability of the microbe to **consume** these nutrients.

Now, how we study the survival and growth of a bacteria?

A: Simply, by studying isolated bacteria that was grown under optimal condition in laboratories (providing the bacteria with excess nutrient and study how bacteria consume these nutrients).

Q: What do we mean by **Bacterial Growth**?

A: Cell Multiplication by a process known as **Binary Fission** that leads to an increase in the number of single bacteria making up a population, referred as culture. So, bacterial growth is mainly by an increase in number **Not** in size. (Note: human growth is adding up biological molecules, building up more proteins, lipids, etc. Ex. Increase in muscle size)

**Binary Fission**: is a process of replication in which one cell (parent cell) splits in half to become two identical daughter cells. How it occurs?

1 – DNA replication (chromosomes must be duplicated)

2 – Elongation of the bacterial cell so the two copies of DNA will go to opposite poles. As we know bacterial cell lack spindle fibers so the separation of the two copies achieved by attachment of each copy to a certain spot on the plasma membrane

3 – Inward growth of the Plasma membrane and cell wall forming a septum between the two copies of DNA. (this invagination occurs through guidance of certain proteins)

4 – Complete splitting will result in two bacterial cells

Note: there is a chance that the two daughter cells stay attached to each other after dividing, this results in grouping of bacteria: For example, in pairs or chains. This is the reason why there is different arrangement of bacteria.

The time it takes for binary fission to occur is called **Doubling time** which varies from one bacterial species to another and also depends on the growth conditions. Doubling time ranges from minutes to hours: For example, E.coli DT is 20 minutes and that for M.tuberculosis can be up to 40 hours .

The idea of Binary fission and doubling time leads to **Exponential Growth** of bacteria which means: **The biomass of Bacteria (B) doubles with each doubling time**.

For example: E.coli doubling time is 20 m, so if there is 2 E.coli in an optimal media, they will become 128 cells after 2 hours ( 6 duplication occurs : 2-4-8-16-32-64-128).we can conclude from this concept the equation of Exponential Growth:

$$N = N_0 * 2^{(t/td)}$$

Where N: number of bacterial cells after specific time

N0: initial number of bacterial cells

T: time elapsed td: doubling time

Now, let's consider the previous example and imagine that we left the E.coli for 24 hours , mathematically 72 duplication will occur and the number of cells would be 472,236,648,2869,645,213,696 This number is only theoretical because growth is limited by two factors: **Amount of Nutrients and formation of waste products.** This means after a certain period of time cell will not be capable to grow anymore,
also some cells will start to die because of the toxicity of waste products.

## Bacterial Growth curve:

It is a curve that reflect the events(phases) that occurs in a population of bacterial cells. It studies the cell concentration over a period of time. There are 4 phases that each population of bacteria encounter during growth:


**Lag Phase**: In this phase the bacterial cell can't grow because the medium is not favorable (growth rate is zero). Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume. We can say it is the time required for transition from unfavorable conditions of growth to favorable ones.

**Exponential Phase**: cells are in steady state and capable of growing. They start binary fission and cells are produced in constant rate. This continues until one of two things happens: either one or more nutrients in the medium become exhausted or toxic metabolic products accumulate and inhibit growth. For aerobic organism, the nutrient that becomes limiting is oxygen.

**Stationary Phase**: cells start to die in the same rate cells are being produced, results in a plateau in the graph.

**Death Phase** (decline phase): only death of cells occurs and there are no cells can undergo binary fission. The rate of cell death is much slower than that of exponential growth phase that is because some cells have adapted with the new environment (they still can't divide but they can survive).

## Cultivation of bacteria

Cultivation is the process of propagating the organisms by providing the proper environmental conditions, such as:

1– **Nutrients**: each Bacteria has a specific compensation of nutrients that provide the building blocks and energy required for growth. For example: Nitrogen is needed to build protein, phosphorus is needed to build ATP, Minerals are essential for enzyme activity. There is a chance that the bacteria is provided with all necessary building blocks but it still can't grow because a certain mutation in the bacteria inhibits the pathway for synthesizing a certain macromolecule: for example, the bacteria can't synthesis amino acids that are required for protein synthesis, in that case amino acids become a **Growth Factor** that must be supplied in order for that bacteria to grow.

2– **Optimal environmental conditions** (PH, salt concentration, Temperature and aeration): each Bacteria has its own optimal conditions, most of the bacteria in our body has an optimal temperature between 30 and 40 degree, these optimal condition can be set on the lab using incubators.

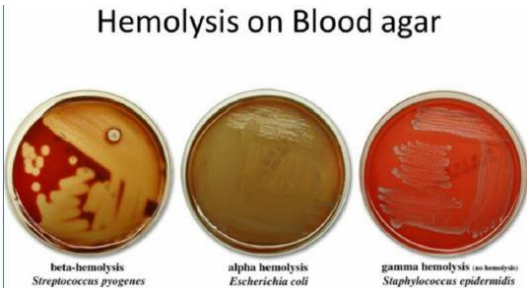
**Note**: The media could be liquid or gelled (using Ager)

**Types of Media for growth**:

1– **Selective media**: it is used to study one type of bacteria by eliminating (or reducing) other types of bacteria that are not in interest of study. This is achieved by allowing only the bacteria in interest to grow either by using Nutrients that are selective for that bacteria or applying antibiotics that kills other types of bacteria but it isn't harmful to the bacteria in interest.

2– **Differential media**: It is used to detect the presence of a certain type of bacteria according to some characteristics (ex: color) that appears due to specific reactions between one type of bacteria with the media. For example: patterns of hemolysis in ager medium containing RBC.

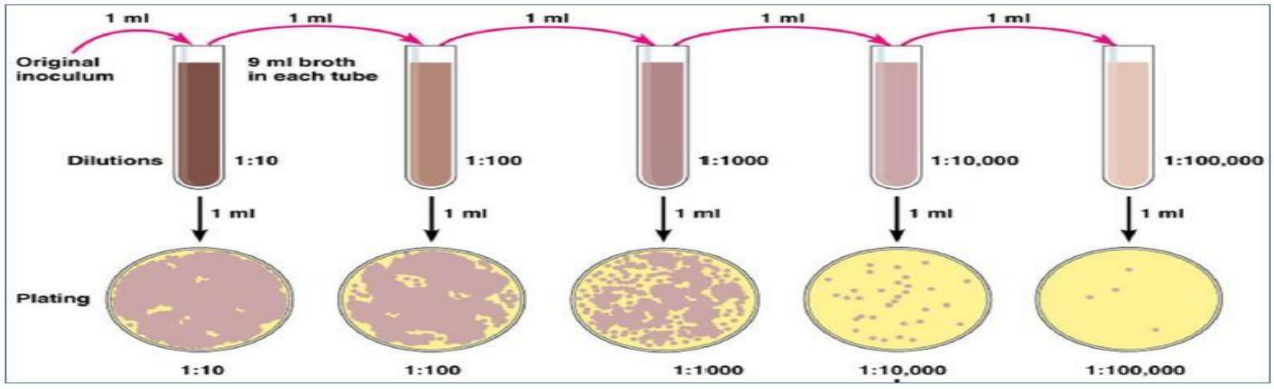
In the picture, each pattern of hemolysis detect the presence of certain type of bacteria.



**Measuring Bacterial growth**: This measurement is done to know two main things:

1– **Cell concentration**: the number of bacterial cells per unit volume of the culture. It can be known by two process:

A– **Viable cell count**: Bacterial viable cell counts can be represented using colony forming units, or **CFU** (assuming each colony on agar started from a single cell). In order to be able to count discernable (separated) colonies on agar, dilution of the sample is often necessary (by decreasing the number of cells in a sample so the colonies can be counted). This can be done by taking 1 ml of the original sample and adding it to 9 ml of water (or bacterial medium), then another 1ml is taken from the new sample and it is added to 9ml of water and so on. Each time the sample is 10 times diluted (the number of bacterial cells is decreased 10 times in each step). After dilution is done, 1 ml of of the appropriate dilutions is put on agar, and the number of colonies on the plate are counted.



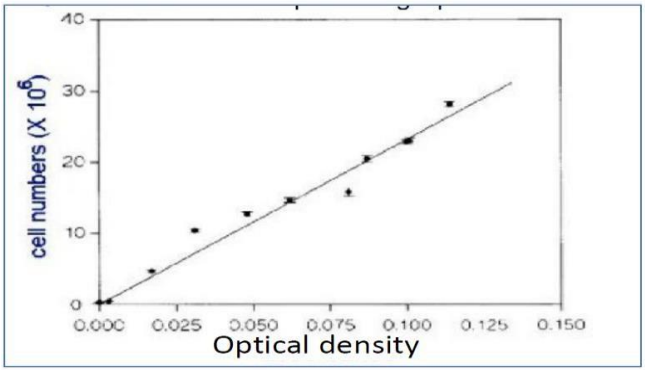

in order to know the original number of cells (in 1 ml of the original sample), we look for a countable plate and we follow the following equations: Plate count * the dilution

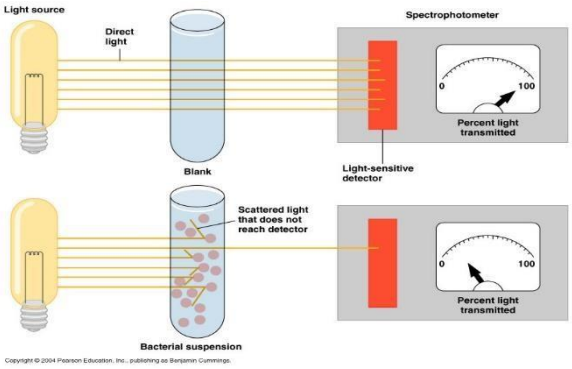
For example: in the picture we can see 4 bacterial **CFU** in the plate after $10^{\wedge 5}$ dilution, so we can estimate that the number of cells in that **sample** to be (4 CFU/ml) and the previous one to be (40 CFU/ml) and in the original sample to be ($4*10^{\wedge 5}$ CFU/ml)

B– **Turbidity**: is a word describing how light passes through a sample of liquid as a measure of how many particles are suspended in that liquid. Microbiologists use turbidity as a measure of cell density within a culture sample. They use machines called photometers and spectrophotometers that shine different types of light through culture samples to determine turbidity. The general assumption is that the higher the turbidity,

the higher number of cells within the culture. This method isn't accurate because the turbidity of the sample may result from dead bacterial cells, so in order to minimize the error, the sample of bacteria must be in the exponential growth phase.

This method can be related to viable cell count using standard curve by plotting a graph (x axis : turbidity (optical density) , Y axis : Number of cells (known from viable cell count method)). Now each reading of turbidity can be correlated to a number of cells.




2– **Biomass density**: it can be measured directly by determining the dry weight of microbial culture or indirectly by measuring important cellular component such as protein concentration (measuring protein concentration is a way to measure the metabolic activity of the bacteria).

Finally, the decision to choose which method to measure growth, depends on what you're studying: so if you're looking for how many viable cells there are you can use viable counts. If you are looking for an expression of protein or metabolic activity of the culture, you can use biomass density.

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**Isolation of Bacteria in a pure culture**

To study the properties of a given organism (ex: antibiotic sensitivity, biochemical properties) we need to study it in isolated culture, because samples from the environment and from patients usually contain a group of bacteria (for example, pathogens and non-pathogens are commonly found in throat, sputum, skin and stool samples).

Firstly, selective medium (discussed above) can be used to limit the types of bacteria growing on agar. Other non-selective, or enriched medium that can grow a group of bacteria can be used as well (for example blood agar).

Secondly, after a variety of colonies appear on agar (mostly with non-selective media), we select single colonies to propagate again and study (sub-culture). Since different bacteria give rise to colonies with different shapes, this helps in choosing the colony to sub-culture.

But samples without dilution often contain many bacterial cells and colonies can not be told apart, to obtain agar plates with single colonies we can select, we will use methods that mainly dilute the sample (ex: pour-plate and streak-plate methods) which aim to isolate single colonies which will be used to grow a pure culture.

1– **Pour-plate technique**: it follows the same method of dilution used in viable count method (discussed previously). In this technique 1ml taken from the sample is mixed with Agar and then left it to solidify. The purpose of Agar is to trap the bacteria.

2– **Streak-plate technique**: the original sample can be streaked on an ager plate using inoculating loop, as streaking continues, fewer and fewer cells are present in each streak and finally a streak may deposit a single colony on the ager. This method only works if the inoculating loop is heated after each step to kill the bacteria stacked on its tip.