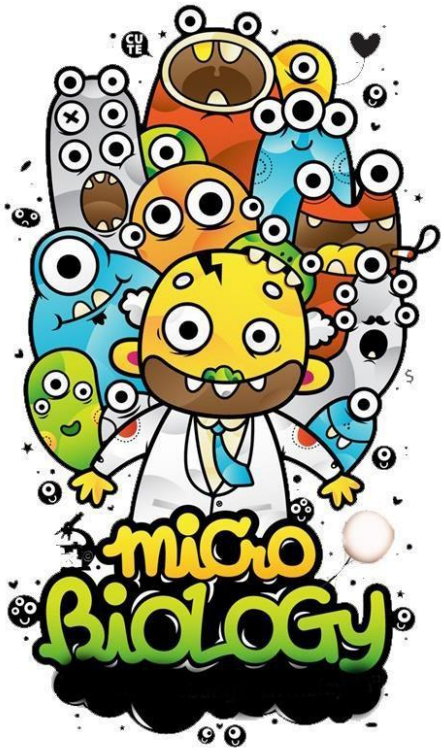

Subject: Bacterial Growth 2

Lecture No: 7

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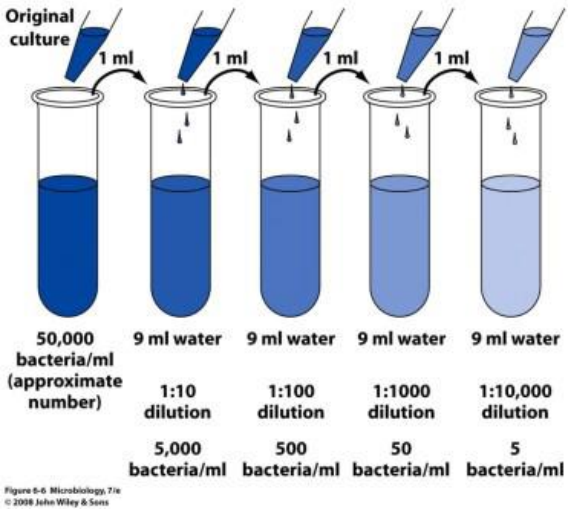
What does bacterial growth mean?

The increase in the number of bacteria.

How do we count the number of bacteria?

1. Serial dilution & plate count method.
2. Direct microscopic count.
3. Filtration method.
4. Othermethods (gas production/ acid production/ turbidity).

1. **Serial dilution and plate count method:**
   -principle: we spread the bacteria over an agar plate and only viable (living) bacterium will be able to form a colony. In this case, we assume that every colony came from only one single living cell and we call it (colony forming unit-cfu). This method is useful when the sample contains (30-300) cells.
   Why not less than 30 cells?
   Because we want it to be statistically **<u>representative</u>** so very low numbers are unacceptable.
   Why not more than 300? Because this would reject the assumption we have made (we assumed that every colony arises from a single cell). This way the aggregation of bacteria will cause a single colony to arise from more than one single cell and we will have continuous growth; therefore, the count won't be accurate.
   ***Agar plate:** Petri dish containing nutrient medium solidified with agar.
   now what should we do if we have a high number of bacteria (over 300)?
   We need to make a serial dilution: a series of dilution to decrease the number of bacteria per ml. (We do ten folds dilutions until the number of bacteria is (30-300)).



After we do the serial dilution we transfer 0.1 ml to the agar plate by one of two methods:

a) spread plate method:

0.1ml sample is placed on the surface of cool solidified agar medium. The sample is spread evenly, then we incubate it and the colonies will apear only on the surface.

b) pour plate method:

we use this method if the

(1)bacterial cells can't tolerate oxygen levels in the air such as microaerophiles which is a type of bacteria that needs oxygen but poisoned by high concentrations of oxygen.

(2) if we want to use more than 0.1 ml of the sample to be more statically representative.

we add 1ml diluted culture from serial dilutions to melted nutrient agar (the agar tempreture must be less than 90 degrees so that we don't kill the bacteria, but we want it to be liquid too, so we add the sample before it soldifies, that's about 45 degrees) mix, then pour in empty plate. We wait until the agar cools down and soldifies; we incubate it. The colonies will appear both within the medium and on the surface of the medium. The disadvantage of this is the damage caused by the heated agar, also the colonies within the medium are smaller than the colonies on the surface.

Notes:

-Actual number of cells=number of colonies on plate x dilution factor.

-This method only counts viable (living) cells.

-The concentration of bacterial cells in the original suspension (culture) is calculated from the number of colonies and is expressed as cfu/ml.

-To improve accuracy: shake tubes before sampling & make several plates from each dilution.

– Weakness of the process:

Doesn't count the cells that <u>died</u> by the time of plating & does not include microorganisms that <u>cannot grow</u> on the utilized growth medium.

2. **Direct microscopic count:**

-In this method a known volume of medium is introduced into specially calibrated etched glass slide called counting chamber (similar to hemocytometer), this slide contains very small wells that will be filled with a known volume (the manufacturer of the slide tells us that each well is 1 microliter for example). So, we can easily count the bacterial cells in every department (and we usually count more than one and take the average). The concentration of the bacteria will be (cells/volume).

-notice that we call them cells here not colony forming units.

-we count viable and non-viable cells in this method so it is less accurate than Serial dilution and plate count method.

-advantages: easy, takes much less time and not growth based (so we don't need to know the exact conditions that are needed for the bacteria to grow).

-disadvantages: we can't use this method with **very low numbers** (a sample containing 10 bacterial cells per ml for example) because the chance that we count them is very low because of the very limited area. So this method is useful only with high density bacterial samples. **The bacterial suspension should be homogeneous** and you **cannot distinguish between living and dead cells.**

So what method will we use to count such low densities of bacteria?

3. **Filtration method:**
   known volume of fluid (i.e. water or air) is drawn through a filter with pores smaller than bacteria (e.g. 0.45µm) -> filter is placed on solid medium (agar plate) -> incubate -> count the no. of cells in each plate -> calculate the number of cells per unit volume (e.g. 100 ml or 1 L)
   Notice that it is NOT a sampling process, the whole volume is taken so every cell will be counted and we don't need the number to be greater than 30.
   We usually use this method to check if a sample is sterile.
4. **Other method:**
   *** Turbidity (the more growth the more turbidity)**

-We can use turbidity to test antibiotics, we use the antibiotic on the bacterial sample and if it increases in turbidity, it would mean the antibiotic is not effective on this type of bacteria (qualitative method). We can use turbidity as a (quantitative) method by using optical density of the liquid as a measurement as well (we make correlation between optical density and the number of bacteria using calibration curves).

***Gas production: can be detected by capturing the gas in small inverted tubes**

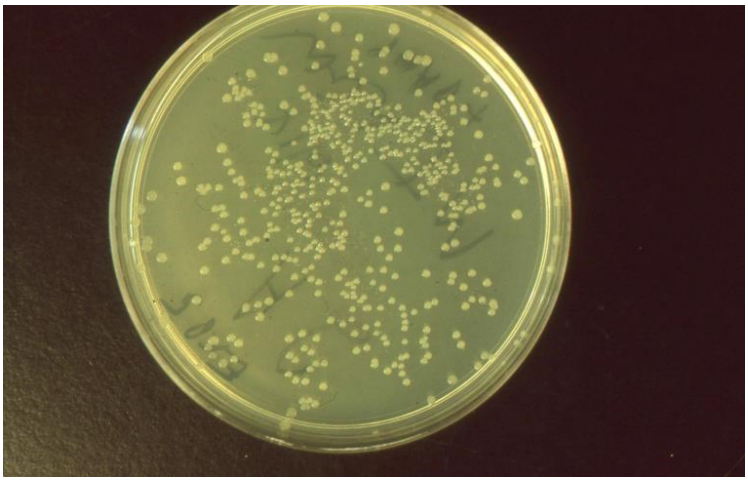
*** Acid production: by incorporating pH indicators (the metabolism done by bacteria usually lowers the pH)**

Culturing Bacteria

What do we mean by culturing bacteria?
Growing bacteria in an artificial environment (Lab).
-when we want to study bacteria the sample must contain <u>the desired bacteria only</u>.
For example: if we want to study pathogenic bacteria in the GI tract and we took a sample from the intestine, we will get a mixture of household bacteria in the GI tract and the pathogenic bacteria, we must purify the sample to get only one type of the bacteria so that we can study it.
Pure culture-> single type of bacteria.
To obtain a pure culture we usually use a <u>solid growth media</u>, why?
(1)It doesn't melt below 95°C, and after melting, it solidifies at ~40°C (hysteresis)
(2) Inert substance: only very few organisms can digest it
(3) So bacteria can form colonies, which are highly characteristic for every type, so if we know the morphological characteristics for the colony of a specific type, we can isolate that type and get a pure culture of that kind of bacteria.
***Note: if we don't know the morphology of the colony, we examine all the types under the microscope to identify them.**

***Methods of Obtaining Pure Cultures:**
1) pour plate method 2) streak plate method

1) pour plate method:
– Makes use of serial dilutions so that the final dilution contains about 1000 organism.
– 1ml of this dilution is then placed in 9ml of melted agar medium (at 45°C) & the medium is quickly poured into a sterile plate.
– The resulting plate will contain small no. of bacteria some of which will form isolated colonies on the agar.
– Since some microorganisms are embedded in agar medium, this method is useful for growing microaerophiles that cannot tolerate exposure to atmospheric levels of oxygen.
****Every colony has arisen from a single cell; therefore, it contains one type only.**



2) streak plate method: (does not require serial dilution)
-Pick bacteria on sterile wire loop (buy dipping the loop in the mixed culture).
-Move the wire along the agar surface depositing streaks of bacteria on surface (do not streak all the plate at once, just streak less than ¼ of it in the first time).
-The bacterial density of those streaks will be very high, that will result in continuous growth of the bacteria after incubation.
- We flame the loop in order to kill any bacteria from the first time.

-Pick a sample from the bacteria deposited on the agar plate and streak again on a much larger area (this decreases the bacterial density so it is considered as a type of dilution).

-Fewer bacterial density means increased chance of getting discrete colonies.

- Flame the loop and repeat about 4 or 5 times (dilution).

- We will get discrete colonies in the regions streaked last.

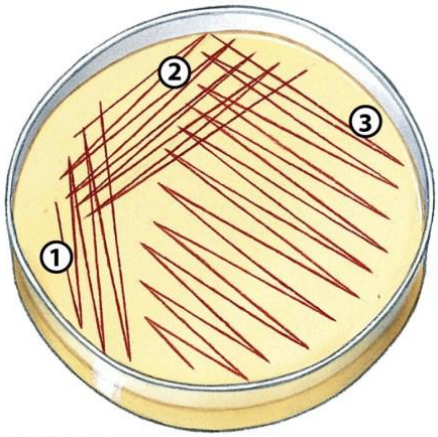


Figure 6-19a Microbiology, 7/e
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Notice that every feature of the colonies is characteristic except for <u>size</u>, it only represents growth, so we can have a pure culture with different sizes of colonies.

Growth media:

-We must supply bacteria with nutrients, oxygen, optimal pH and temperature in order to culture it.

-We give bacteria nutrients through culture media (Although many bacteria can be grown in the lab nowadays. Some microorganisms, such as those causing syphilis & leprosy, still cannot be cultured in lab media but rather need cultures containing living animal cells).

Types of growth media (according to the physical state):

1- **Liquid growth media (broth):**
   -nutrients that are dissolved in water (aqueous solution).
2- **Solid (agar) growth media:**
   -if we added agar to a liquid growth media it would act as a gelling material and will solidify it.

Note:

***that every Liquid media has a corresponding agar (solid) media.**

***agar is a gelling agent not a nutrient, so bacteria cannot metabolise agar (this is important because we want it to stay solid).**

Types of growth media (according to the composition):

1- **Defined synthetic medium (chemically defined media): Synthetic medium that contains specific kind & amount of chemical substances. (Few nutrients/ suitable for few types of bacteria).**
2- **Complex medium (chemically non-defined medium): Contains reasonably familiar materials but varies slightly in chemical composition from batch to batch; for example, the blood. We know that the medium contains 1 gram of blood, but the blood is from a complex medium and contains thousands of chemicals, so this type of media is not chemically defined.**

Examples: Blood/peptone/extracts from beef/yeast/soy beans/etc.…

-Peptone is animal proteins that has been degraded by enzymes to smaller peptides.

-Yeast extract: contains a number of vitamins, coenzymes and nucleosides.

-Casein hydrolysate: made from milk protein andcontains many Amino acids.

-Blood (or serum): contains many nutrients needed by fastidious pathogens, Blood agar (usually sheep's blood) is also used to identify microorganisms that cause hemolysis.

Complex media can support many types of bacteria at the same time (much more than the chemically defined media).

Types of growth media (according to the purpose):

1- **Lab media (supports bacterial growth).**
2- **Selective growth media (support specific types of bacteria only).**

3- **Differential growth media (support bacterial growth and differentiate between deferent types of bacteria).**
4- **Enrichment growth media (support bacterial growth but may enhance the growth of one type more than the others).**

***Please check the slides for figures.**