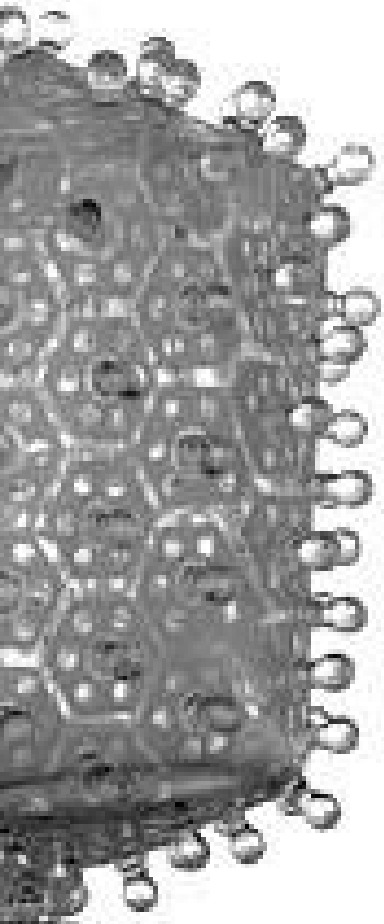


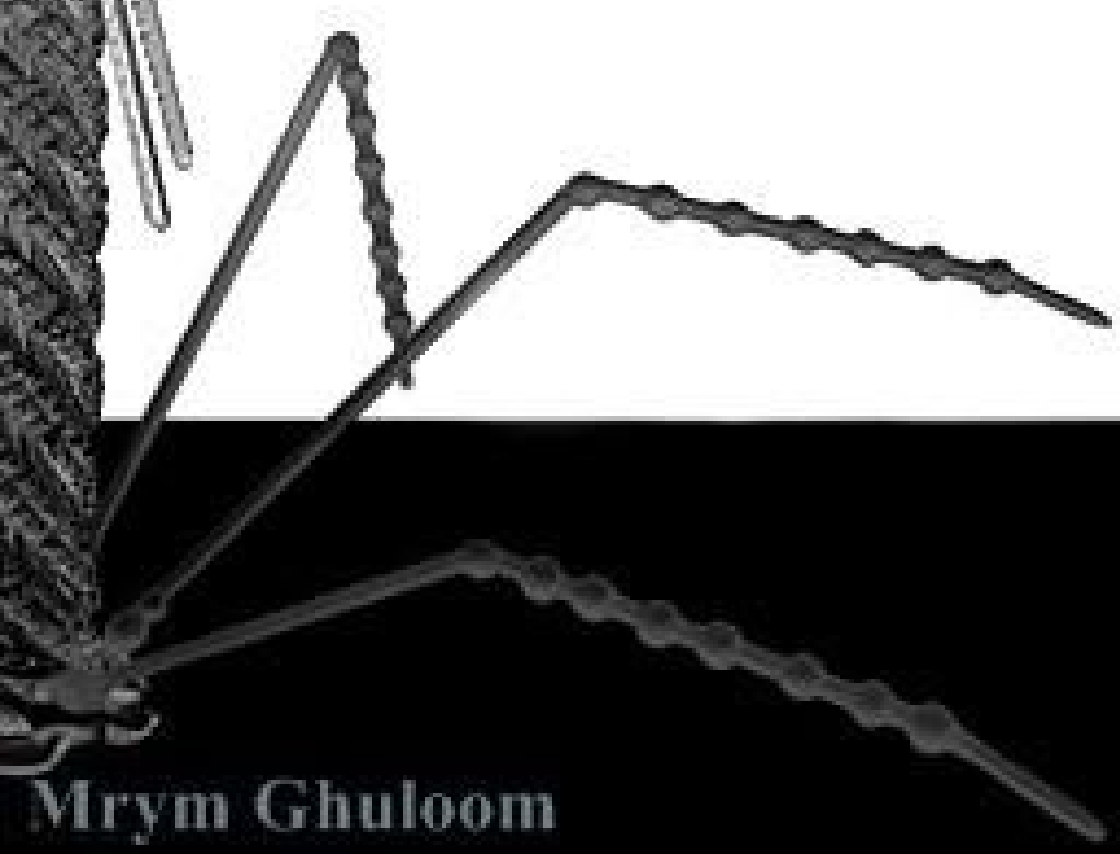
Microbiology

Lecture No: 4

Dr Name: Asem Shehabi

Done by: Rahaf Qasm

Sheet ■ Slide □



Mrym Ghuloom

Virulence factor :

What does it means? We have various types of bacteria, some are pathogenic, some are more pathogenic, some are toxigenic , other non-toxigenic...etc.

In order to know the difference between various types of bacteria we have to know what those types of bacteria produce of specific types of enzymes, toxins, or other fragments and appenditions as adhesion proteins in relation to fimbriae , to pili ... any of these harm factors might contribute for enhance of pathogenicity of bacteria and might be associated with development of clinical case and infection whether in our respiratory path or skin or other parts of body .. Each bacteria has specific harm virulence factors .

Keep in your mind that these virulence factors can be recognized during host infection in form of septicemia , bacterimia or other features ..skin rush or severe inflammation...etc, and most of these virulence factors are associated with development of what we called specific Antibodies because these virulence factor s are antigens and once are hosted or contacted with immune system, they are responded to by production of immunoglobins (antibodies).

Generation Time:

The generation time differs from one type of bacteria to another:

- The rapidly growing facultative anaerobic bacteria which are normally associated with a majority of infection in our body specially in relation to the bacteria which are found in the intestines and respiratory tract: the generation time of it is always between (20 – 25) minutes.

- certain type of bacteria like **M-tuberculosis** has a generation time of about (1 – 3) hours

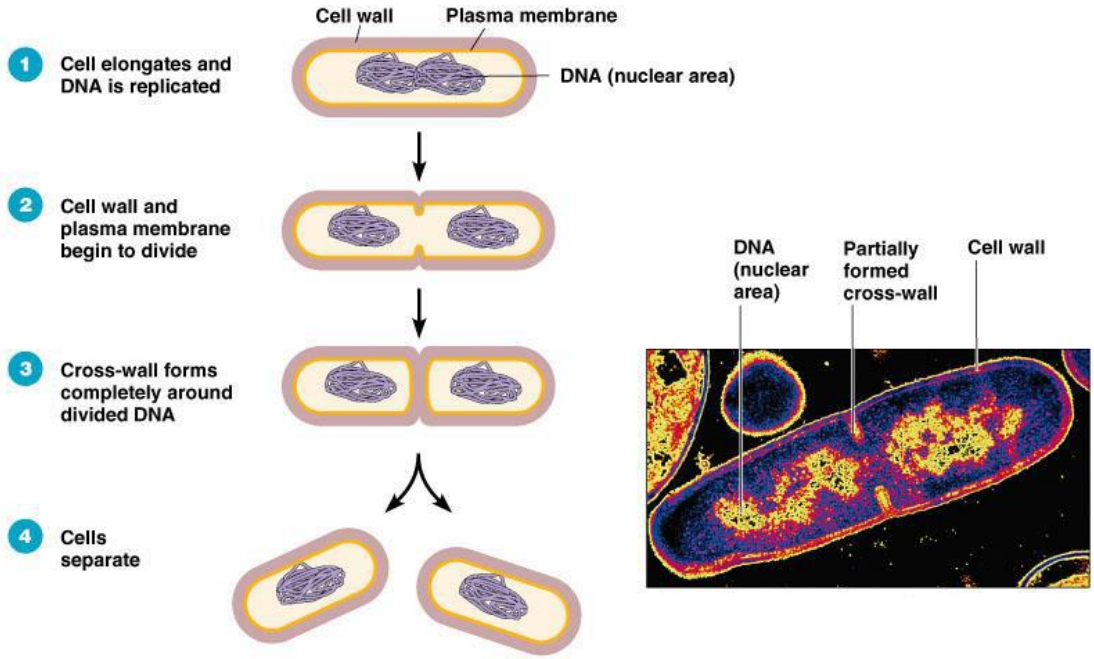
During the growth of any cell :

Firstly the cell must be biological active : (which means it must prepare enzymes in necessary for replication. and later on there will be three or four steps which result in:

- conservation of the DNA , separation the DNA into two equal parts. chromosome usually will be open and each copy resulting in developing of double strand DNA (chromosome)

Later on separation will be followed by invagination of cytoplasmic membrane and cell wall from outside to the inside as you see in picture above now we have two separation cells.

As in this picture:



(a) A diagram of the sequence of cell division.

(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

In the laboratory in order to propagate and to increase the number of cells and recognize the presence of the colonies we need about 18 hours; in this period any single cell will reach (10 ^6-10^8), and this is a huge number in closed-growth system not in an opened-growth system like in our industry or in our intestine tract cells, in fact our intestine tract is devided between an closed- and opened-growth system, we will talk about this later on.

There is no growth of bacteria without presence with essential supply of nutrients , source of carbon, nitrogen ....etc and presence of oxygen in obligate aerobes. In addition to minerals , vitamins ....etc.

we classify bacteria into different group according to their need for oxygen :

1-obligate aerobes : can't grow without presence oxygen

2- facultative anaerobes : which can grow in presence or absence of oxygen

3-oligate anaerobes

4- A subgroup called Aerotolerant anaerobes

Why is it important to have aerobic organism which requires oxygen in respiration during biochemical oxidation-reduction system ?

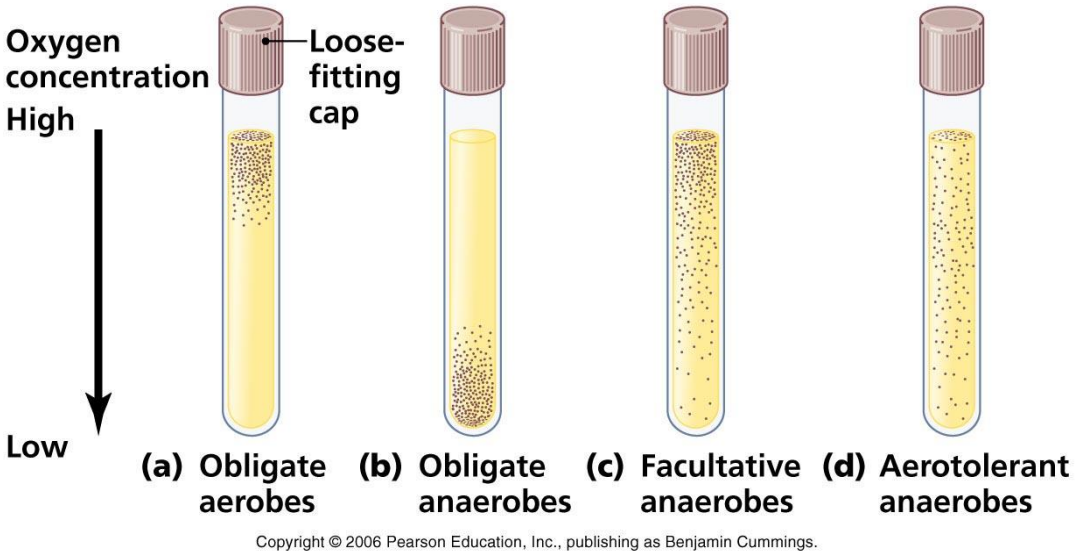
Oxygen is a toxic material .. specially if there is radical oxygen with active electron so it might produce damage to cell membrane and might inactivate enzymes which are necessary for growth of bacteria and for the transfer of nutrients.

Therefore aerobes and facultative anaerobes must contain at least two of three enzymes in order to get rid of the radical o:

Generally superoxidase dismutase and catalase are enough but there are some bacteria have the three types together superoxidase dismutase + catalase+ peroxidase

Catalase alone isn't enough .. peroxidase alone also isn't enough..

so as you see in chemical reaction in slide (24) we have at least two enzyme in order to get rid from radicals allow oxygen to participate in the oxidation-reduction system of material cell and to supply energy necessary transfer of nutrients and molecules for growth of bacteria.



We note that aerobes try to live near the surface where there is more oxygen and anaerobes at the bottom where there is lack oxygen and facultative are distributed in whole the tube but concentrated at the top like in our intestinal tract: first part of colon and last part of our small intestine

there we have facultative anaerobes and more obligate anaerobes. In colon we have more anaerobic conditions so only anerobes can survive. Aerotolerant anaerobes can survive for few minutes outside the body.

But we must know that facultative anaerobes is the most important in relation to pathogens (up 95 % of whole human infection are related to facultative. however 5-10 % is related to obligate anaerobes and in certain cases we have mixed infection compose of both facultative and obligate anaerobes specially in intestine tract, mouth and vagina) the concept that some physicians tell that facultative is predominant in our intestine is fault .. 95 % of intestinal flora compose of obligate anaerobes and only 5% or less of facultative bacteria

Note: under certain conditions the infection is related to obligate anaerobes such as intestinally infection related to rectum.

These bacteria in our oral cavity and in vagina are very important to maintain the right conditions for these regions ***example*** :

Lactobacilli in young ladies presence in vagina to keep the PH of vagina acidic and this means to prevent developing of infection specially during sexual contact. Otherwise PH of vagina will be alkaline associated with infections with bavteria as well as with fungi.

Bacteria classified by the source of their energy , oxidation-reduction process into two groups :

Heterotrophs : type of bacteria which should be supplied with complex organic compounds example : sugars ,proteins , source of carbohydrate ...etc

Autotrophs :Fix carbon dioxide to make their own food source

survive by fixing carbon dioxide or nitrogen and often they require for growth the source of energy other than oxygen (inorganic compounds)

survive in nature , not pathogenic

They are rarely associated with infection.

The source of energy for **photoautotrophic** is light energy in oxidation-reduction reaction light

Chemoautotrophic : Instead of light they use nitrogen or sulfur or other minerals during the oxygen process to allow the electron flow process

developing carbon cycle, sulfur cycle which ends usually with production of unit of proteins and sugars necessary for growth.

Saprophytic bacteria:

Non –pathogenic bacteria, take their energy throw fermentation of sugars , the end product of fermentation are : lactic acid , alcohol ,..... etc they also are used in the process of carboxylation.

Found in the nature specially in vegetation environment, soil ..

Keep in your mind that saprophytic isn't autotrophic, it is similar to facultative, under certain condition it may reach our body and produce infection but they are not obligate, like commensal bacteria which is adaptive to our body where saprophytic is adaptive to environment but might contaminate....

Such types of bacteria can't produce infection, their end products form toxics before other substances might produce endotoxification , might produce some side effects in our stomach . If we have type of food as cheese contain large number of saprophytic bacteria might produce large amount of protein, sugars and produce end product called **Protamines** (I am not sure of spelling)with side effect,,, but it will not kill us , it will only produce uncomfortable condition in our body

Culture media :

We will hear about types of culture media used for Isolation ....

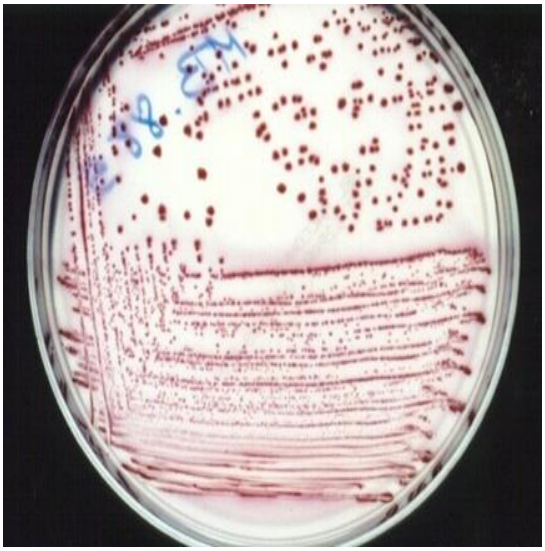
What is culture media means?

Nutrition media contain necessary compounds for growth of bacteria.

In general nutrition should contain carbohydrates and proteins at least. In addition it may contain blood (from human & animals) , minerals (sodium chloride , Mg, K ..), water (very important)

PH is very important; different types of bacteria grow under different PH conditions and Temperature also: there are various types of bacteria grow over earth with wide range of temperature

As you see in next picture:



Growth culture:

Broth culture (fluid medium)-

Solid cuture (Agar) , blood agar , nutrition agar-

Here is an example prepared of MacConkey Agar (solid medium) containing a specimen may be urine affected with large number of colonies , each colony is composed of at least 10,000 cells in general. As you see there is no separation of colonies, we call this confluent growth

On right there is a test tube (liquid medium). Usually colorless if there is no bacteria, if there is bacteria it will produce turbidity.

Types of culture media:

General culture media: support in relation to common infection to human,

If you take a blood from patient infected with unknown organism . it can be gram –ve or gram +ve, it can be facultative anaerobes or aerobes for any type of bacteria we have to use a special culture media, we use nutrient agar , blood agar , chocolate agar .. this media it will support at least one hundred types types of bacteria often associated with our body.

If we have patient suffering from diarrhea and we want to know the causative agent of diarrhea if it is shigella or Salmonella or ... we have to use selective media specially to detect it .

Selective media: medium which supports only certain causative agents of disease particularly in relation to diarrhea or in relation to small-forming bacteria and so on

Selective and differential media: these are between 100 % or 99 % selective and differential media.

Supports the growth of certain gram positive or gram negative

Example : in relation to the urinary tract we often used we use **MacConkey agar** which is composed of bile salts (related to gallbladder) , lactose , neutral red dye and few complex organic compounds .

It used fistly to recognize usually gram –ve then to recognize lactose fermenantors

In slide 31 we have seen that colony will be colored in red due to fermentation reaction of lactose and change of color of media according to PH of medium.

These are examples how we can manipulate the type of media to recognize the causative of the disease in order to reduce the time of the biochemical test.

Lactose fermenantor bacteria : example E.coli.

Lactose non-fermenantor bacteria : example : Salmonella , Shigella ...

We have media wich support only gram –ve , other support only gram +ve, and media that supports both.

So culture media is a diagnostic test in laboratory which help you to identify different types of microorganisms specially which produce infection in our body,

These types of bacteria and colors and morpholpgic characters will help us to diagnose and identify causative agents of diseases .

Not all bacteria grow under neutral condition . neutral condition means PH (7 – 7.2)

Majority of facultative anaerobes which produce infection in our body are considered **neutrophilic** bacteria. (Grow best at pH (7-7.2) so it will signigy our using of culture media nd detection of pathogens)

Most of bacteria belong the pathogens go with first group(Neutrophilic bacteria)

Mesophilic bacteria : type of bacteria which grow not necessary at the same temperature, might grow at the same PH & differ from neutrophilic

bacteria which grow in (7 – 7.2) growth temperature almost about optimal temperature (35-37)c this is important for detection of pathogens .

Keep in your mind , during infection we migh have only few cells of bacteria or maybe the pateint is treated with antibiotics so we may not have a large number of bacteria to be easily detected. So we must do the condition which support the growth of few number of cells.

In addition, in comparison with neutrophilic bacteria we have few number of bacteria called (Acidophilic bacteria) which means production acidic condition and often are represented by lactobacilli which are found usually in new born baby. first organism is lactobacilli in intestine in babies , the second lactobacilli resine in vagina of ladies.

It is important in production of very useful end product specially in fermentation of milk, production of yoghurt, cheese...etc . lactic bacteria and many species are very important in the economic of production of their product in relation to our body .

In oral cavity we have lactobacilli but usually an elderly person has less lactobacilli.

There is huge discussion about use brobiotic which is composed of type of lactobacilli which might replace sometimes the using of other drugs specially using antitoxic drug , antimalignant drug and antibiotics bcause there will be change in the intstinal flora and those brobiotics will help to restore normal intestinal flora this is very useful to keep health condition for patient .

Another two types of extreme bacteria of no pathogenic importance

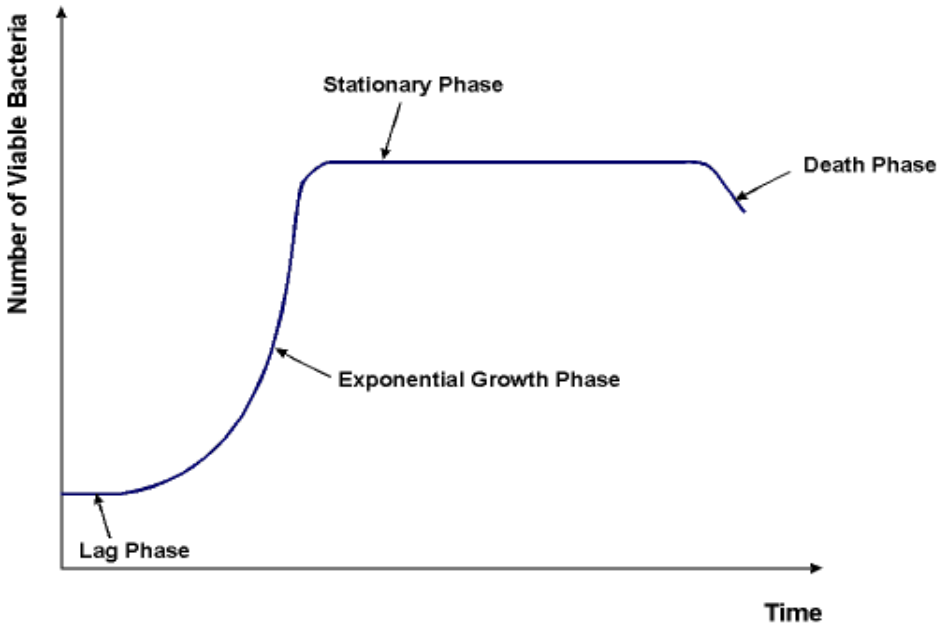
Psychrophilic bacteria : which grow at temperature less than 10 c , found in our refrigerator associated with food.

Thermophilic bacteria : optimal temperature is 60 or above ,found only in hot water and often as non-pathogenic bacteria. Important to neutralizing of sulfur compounds

I have mentioned bacterial growth : one cell become two cells then 4 ... 6 and more ... means production of colonies increasing number of cells . generally facultative anaerobic bacteria within 24 hours produce not 10 ^3 but about (10^6 – 10^7) cells.

We reach one important last stages as you know in human and animals the age is measured in days , weeks , month . years ... but we can't use this technology in bacteria .. in fact we measure this within 24 hours to maximum 96 hours (1-4)days

Bacterial growth curve :



We have four stages of growth for any type of bacteria whether in fluid or solid medium but not in continuous culture , in close culture

Difference between continuous and close culture as an example industry they produce end products of bacteria and fungi using continuous culture.

You control flow of oxygen , minerals , nutrients , control the toxic product .

Continuous culture is more expensive and more difficult to control whereas growth culture we have a petri dish:

You place few number of cells from other cultures or from our clinical sample such as blood, urine...etc.

1 drop about 0.01 ml We place on the surface of medium on the top , and we use pathological loop in order to have a streaking methods to dilute this fluid on the surface of the medium in 3 directions, in order to

recognize at the end we have called a single colony , at the begging we have growth, heavy continuous growth ,less growth and at the end we are using best 3 streaking method; at the end we have single colony which can we utilize it for gram stain , important to be used for biochemical reactions. We can't use mixed culture , because we will have mixed end products , so culture formed for any type of organism in order to be studied and to identify, we must use **pure** culture originated from one single cell not from two cell or more, if from two cells we might have the same culture but we have to recognize that there is a difference between the growth of one colony and another.

Streaking method allow us to know if our clinical samples contain one single organism, two or more .. example : from skin or from urine or from blood.

If we have urine culture with two or three organism often we consider this as contamination , we don't have true type of organism which is the causative agent a certain something due to collection of species not under recommended conditions.

Go back to curve :

There are four phases we have a culture tube usually in fluid medium free of any type of bacterial cells or other viruses ... etc

We place the tube and we place from our start culture which is the plate as you have seen one single colony

We take one single colony and place with the pathological loop in this tube then we Start to observe the growth

How to know the growth ?

Enumeration of cells by direct cell count

After one hour we take 0.1 mm and we look by place on the culture of medium to see the number of cells that come out of it 1000, 2000

You see different growth and plating of organisms, at the beginning we have a huge number of cells , in the second plate we have after 1 to10 D , then there are less cells 1-1000 more less and so on .

At the end (one t 100,000) or(1 to 10^6) we might recognize only few cells . Maximum we must have on the surface of plate 100 colony

In order to calculate later dilution factors which is 2 , 3 , 4 ,5 ....etc

In order to see.

At the end we have this numbers of organisms and this in fact can be translated later as you see later in what we called :

lag phase: which is adaptation phase where cells of bacteria begin to become biological active , to be adaptive to environment , PH , temperature .....

second : logarithmic phase (logarithmic duplications in each cell in thousands)

and after a few hours it depends on the types of the culture medium and types of bacteria we have what we call

Stationary phase : bacteria has slow growth because already huge numbers of cells have utilized available nutrients in the medium , there is no enough nutrients in growth system of medium to continue in continuous culture duplicating.

In this phase : number of new cells is equal to number of dead cells

Stop in growth and later on due to presence of large number of cells in close culture medium we reach the last phase death phase due to activation of autolytic enzymes , majority of bacteria are broken down into small particles

At the end instead of 1 billion bacteria cells we have 1 million for example..

If we stay waitong another few hours we might have all bacteria in the culture medium killed.

to the most wonderful colleagues :

I hope everyone achieves his goal

And ask God to help you and me to succeed and carry the message of Medicine honestly

your colleague :Rahaf kasem