3

Microbiology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

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In the Name of Allâh, the Most Beneficent, the Most Merciful

This sheet is written according to the lecture in section 2. The under lined sentences are not mentioned in the lecture and the slides so they are not required, they are just for more clarification and further underestimation. Some of which are mentioned later in some lectures.

-**Spores :**

~ A spore is a dormant cell " a state of suspended animation" (highly resistant structure) that some bacteria can adopt when conditions are not ideal for growth , such as shortage or lack of nutrients. Spores are analogous to plant seeds and can germinate into growing bacteria when conditions are right.

—They can still for centuries.

They are resistant to Heat,Drying irradiations ,Cold. For instance to heat , spores can still viable in a boiling temperature for one hour of resistance , but only moist heat e.g 120C for 20 minutes will kill them. This is the only way to kill spores which's called Autocleave: it is a process that destroys spores and bacteria. It is done at high temperatures and under high pressures.

Autoclave: is a pressure chamber that is used to sterilize equipment and supplies. When these items are placed inside the autoclave they are exposed to high temperature steam (usually around 120 degrees Celsius) for about twenty minutes

—The location of the spore within a cell is a characteristic of the bacteria and can assist in identification of the bacterium.Central , sub-terminal, terminal. Central endospores are located within the middle of the vegetative cell. Terminal endospores are located at the end of the vegetative cell. Sub-terminal endospores are located between the middle and the end of the cell.

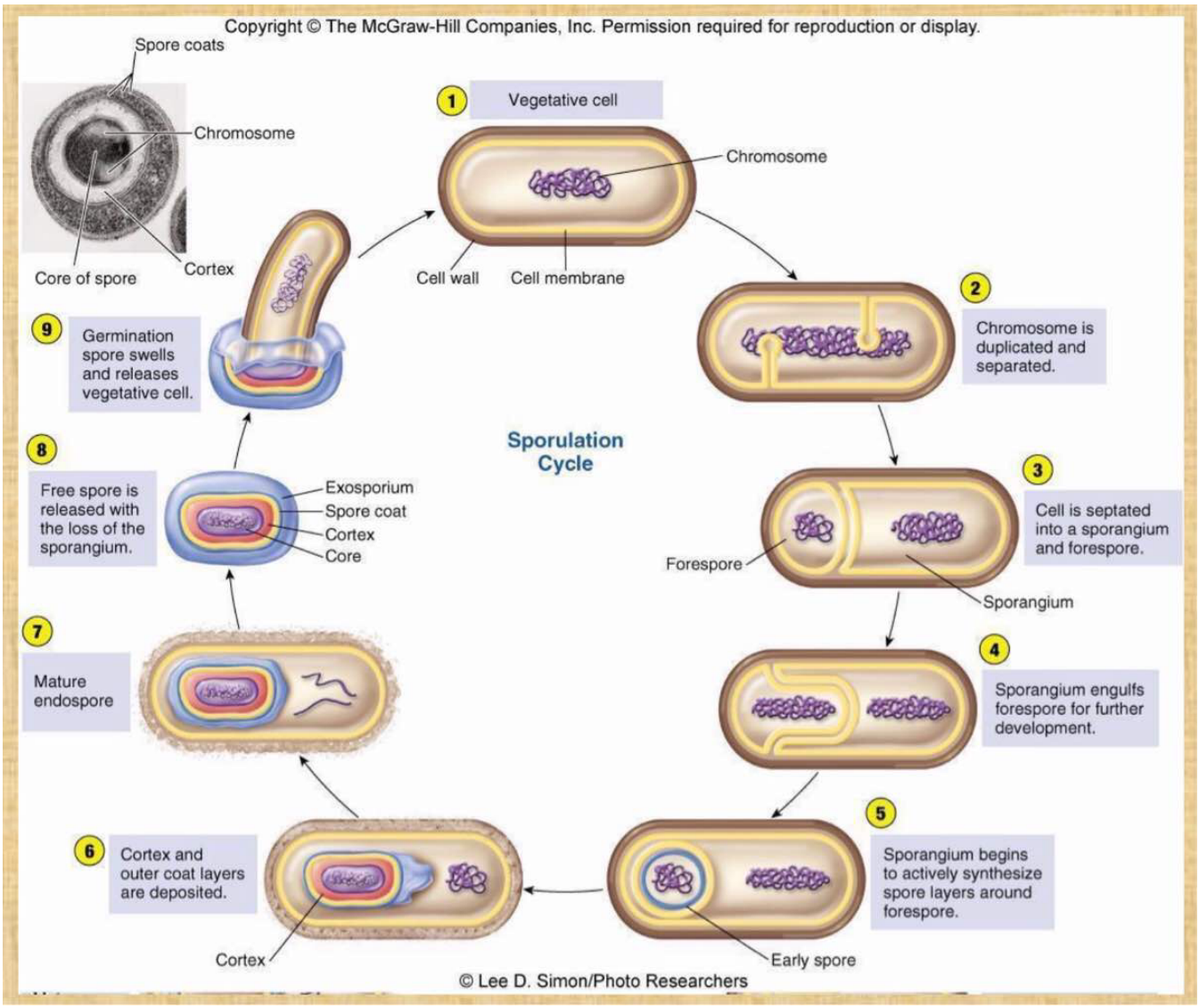
—Two genera of medical importance produces spores : *Bacillus and Clostridium, they are the only two bacteria that can form spores*.

—Contain *Calcium-dipicolinate and Keratin layer.*

—Stained by different stains e.g ZN stain, malachite green.

-Sporulation: is the formation of spores ..and.. Germination: is the sprouting of a spore.

-Formation and circulation of spores



Under hard conditions, which are not ideal for bacterial growth, the vegetative bacterial form will undergo sporulation. First of all the chromosome is duplicated and separated, cell is septated into Sporangium and Forespore, Sporangium engulfs Forespore for further development, Sporangium begins to actively synthesize spore layers around the Forespore, cortex and outer coat layers are deposited that surround the Forespore which will be the future spore/endospore, mature endospore has formed, finally a free spore is released with the loss of the sporangium

*Sporangium

A saclike structure (a cell) within a fungus, in which asexual spores are borne by progressive cleavage.

*forespore

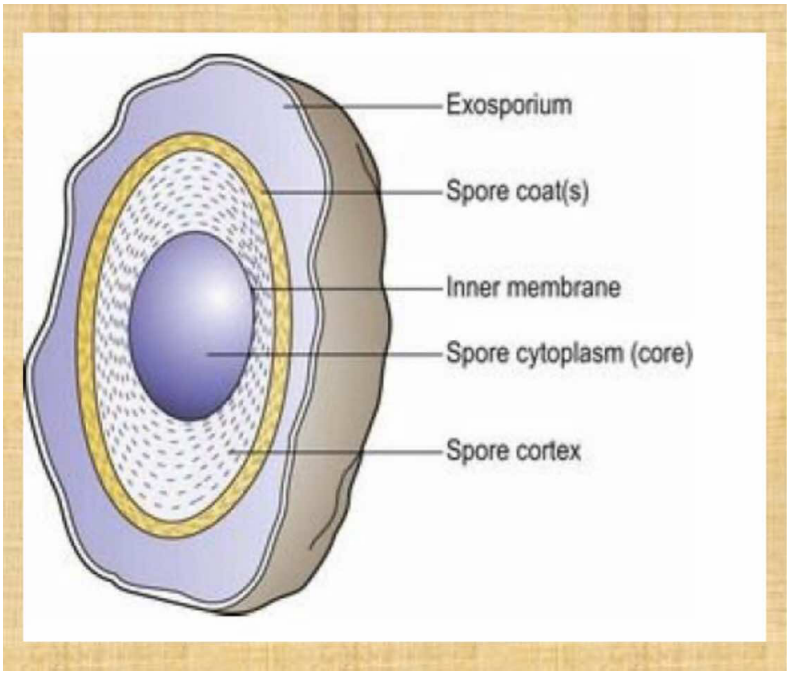
a stage in the process of SPORULATION that can be identified as a refractile body not yet resistant to heat.

-Why are spores more resistant to environmental stresses?

Spores are more resistant because they are not growing; they

are desiccated, and they are covered with multilayers of a peptidoglycan-like material and a keratin-like protein coat.

-Structure of spores



-**plasmid:**

an extrachromosomal circular DNA double stranded molecule self-replicating independently of the essential bacterial chromosome. It is a structure found in some bacterial cells that carries genes for a variety of functions not essential for cell growth(give some properties).Transmissible or non-transmissible, transmitting is through successive cell divisions.

-genes specifying such functions as antibiotic resistance, genes for pili

and virulence factors (e.g exotoxin)

•We can say that **virulence** is how much an organism has weapons *to attack* and *to defend against anything*.

exotoxin

a potent toxin formed and excreted by the bacterial cell and found free in the surrounding medium; exotoxins are the most poisonous substances known. They are protein in nature and heat labile, and are detoxified with retention of antigenicity by treatment with formaldehyde. Bacteria of the genus Clostridium are the most frequent producers of exotoxins; diphtheria, botulism, and tetanus are all caused by such toxins.

-The production of enzymes, toxins and antigens; and the metabolism of sugars and other organic compounds. Plasmids can be transferred from one cell to another by conjugation and by transduction. Some plasmids may also become integrated into the bacterial chromosome; these are known as episomes.

-Plasmids are not essential for cellular survival.

-**Transposons**

Pieces of DNA that moves from one site to another either within or between the DNAs of bacteria, like plasmids and bacteriophages. Sometimes transposons are called “Jumping genes”.

Bacterial physiology, metabolism and growth

Bacterial survival = growth & replication. Can not just sit around.

*Growth stages include metabolism, regulation &division.

Growth needs material (nutrients) and energy/metabolism.All cells require a constant supply of energy to survive. This energy is derived from the controlled breakdown of various organic substrates (carbohydrates, lipids, and proteins). This process of substrate breakdown and conversion into usable energy is known as catabolism. The energy produced may then be used in the synthesis of cellular constituents (cell walls, proteins, fatty acids, nucleic acids), a process known as anabolism. Together these two processes, which are interrelated and tightly integrated, are referred to as **metabolism**.

• Fast growing bacteria that divide each 10-30 minutes e.g Vibrio .

• Slow growing: each 24 hours e.g Mycobacterium tuberculosis.

• Bacteria consists of many structures & elements e.g protein, polysaccharides, lipids, nucleic acid & peptidoglycan.

• Growth needs materials (nutrient) & energy/metabolism

-Mycobecterium which have a very complex cell wall arrangement, are an example of slow growing bacteria: each 24 hours to divide.

•Growth in bacteria is not just increasing in size, it is also in number, so
Bacterial growth : Increase in the size of organisms and an increase in their number.
•These two processes are balanced,the net effect is biomass (increase in the total mass) of the culture.

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The number will be adopted here,it is important as outcome of infections and in the measurement of the effects of antibiotics

-Types of growth in the laboratory:

• In the laboratory, bacterial growth can be seen in three main forms:

• 1.By the development of colonies, the macroscopic product of 20–30 cell divisions of a single cell. That means we are going to see 20 to 30 colonies within a Petri dish.

• 2.By the transformation of a clear broth medium to a turbid suspension of 10^7–10^9 cells per mL.

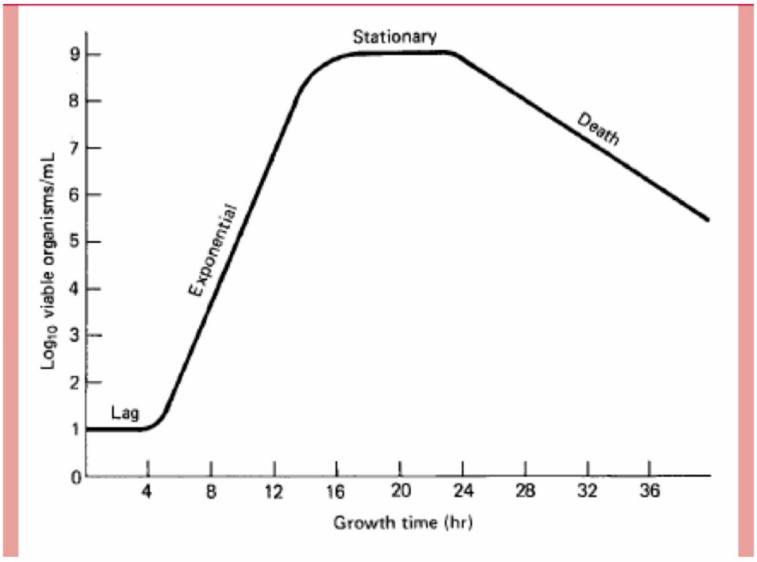
• 3. In *biofilm formation*, in which growth is spread thinly (300–400 µm thick) over an inert surface and nutrition obtained from a bathing fluid

.....

—Biofilm: is a layer of prokaryotic organisms that have aggregated to form a colony. The colony attaches to a surface with a slime layer which aids in protecting the microorganisms. Biofilms often form on the inert surfaces of implanted devices such as catheters, prosthetic, cardiac valves and intrauterine devices.

So bacterial growth is determined by 1- Colonies 2- Turbidity of the clear liquid broth 3- Biofilms just like dental plaques.

-Bacterial growth stages:



For sure there's an overlap between each two phases.
Accordingly, some individual bacteria are dividing (by binary fission) in lag phase, some start to die in exponential phase, stationary phase has equal rate of dividing/growth and death rate (death is due to the lack of nutrients, spreading of toxic substances from the bacteria it self). But briefly talking the points below are the main idea we are considered to know.
#Note: For exponential growth, plotting the natural logarithm of cell number against time produces a straight line.

1. Lag phase, there is little or no change in the number of cells (adjustment stage),but .1 metabolic activity is high. And adaptation is taking place.
2. Log or exponential phase, the bacteria multiply at the fastest rate possible under the conditions provided. The bacterial population doubling occurs at a constant rate.

3. Stationary phase, there is an equilibrium between cell division and death (nutrients start to deplete and toxic materials start to be produced “just like exotoxin”)
4. Decline phase / Death phase, the number of deaths exceeds the number of new cells formed

In this phase sporulation can take place by *Bacillus* and *Chlostredium*.

-Extending log phase:

Chemostat(chemical environment is static): cells of a growing culture are harvested continuously and nutrients replenished continuously.

Maintenance of bacteria in continuous culture is sometimes necessary in industrial and research purpose.

-Media’s used to isolate the bacteria:

Such usage of media’s for culturing : semi-solid or solid media in a Petri dish + Agar and it look like Jello / another media is liquid called “broth” in a test tube . So growth of bacteria depends on the media and the appearance of colonies. Explanation of colonies developmental d types of growth has been mentioned.

#Main features of media in medical bacteriology are:

• 1. a source of protein or protein hydrolysate(hydrolysate are the substances produced by hydrolysis), often derived from casein.• 2. control of pH in the final product (after sterilization). Each bacteria has its own appropriate PH level. • 3. a defined salt content.

In the laboratory :

Culture media: is a nutrient material prepared for the growth of bacteria in a laboratory. Microbes that grow and multiply in or on a culture medium are known as a culture.

Agar is a common solidifying agent for a culture medium. Agar media are usually contained in Petri dishes or test tubes (slant or deep). Tubes are often used for biochemical reactions, to determine the motility , H_2S production and so on.

-Bacterial growth requirements as amount of nutrients and location:

- *Fastidious* organisms they require high amount of nutrients

- *Simple* requirements can make everything from scratch.

- selective (enrichment) with indicator

Some bacteria cannot be cultured in vitro (Lab.). (they cannot be cultured outside their biological context).

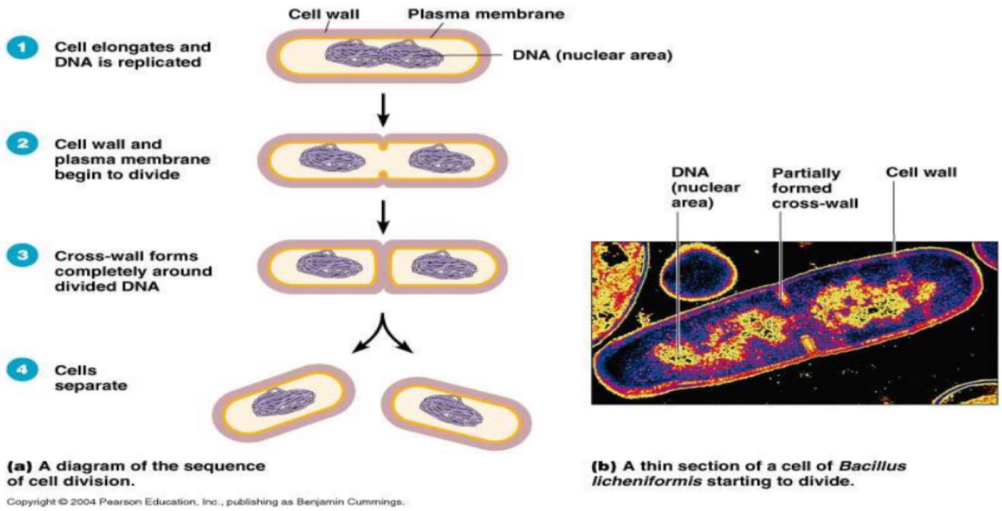
a. Chlamydia and Rickettsia : need tissue culture like viruses.
b. Treponema pallidum, Mycobacterium leprae, require animal infection

We cannot predict the virulence of bacteria by their growth rate ; some slow or non-culturable bacteria can be fatal if infected.

-Bacterial division and generation time:

The reproduction method of bacteria is binary fission, in which a single cell divides into two identical cells. Some organisms (other than bacteria) reproduce by budding “like fungi” aerial spore formation or fragmentation

• Cell division occurs by the development of constrictions mediated by the assembly of an actin-like protein.
• Constrictions proceed from the periphery inwards and, in some cases, produce a transverse cell wall known as a septum or cross-wal.



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

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

-Metabolism of bacteria:

Building up and breaking down processes (metabolism) is so much faster in bacteria than our bodies, that lead to a faster growth. In which bacteria use so many compounds as an energy source. The study of bacterial metabolism focuses on the chemical diversity of substrate oxidations and dissimilation reactions (reactions by which substrate molecules are broken down), which normally function in bacteria to generate energy. Some biosynthetic processes such as those producing lipopolysacharide (LPS / endotoxins) LPS helps for bacterial viability " to keep alive " and teichoic acid , they are unique to bacteria.-Bacterial metabolism involves approx. 2000 chemical reactions. These reactions can be categorized according to their function in the metabolic processes of ***fueling, biosynthesis, polymerization and assembly.***

• Energy Production = Energy Consumption. Because when we want to consume energy, we must had produced energy for this consumption. And if we want to produce energy, then this energy must be consumed.

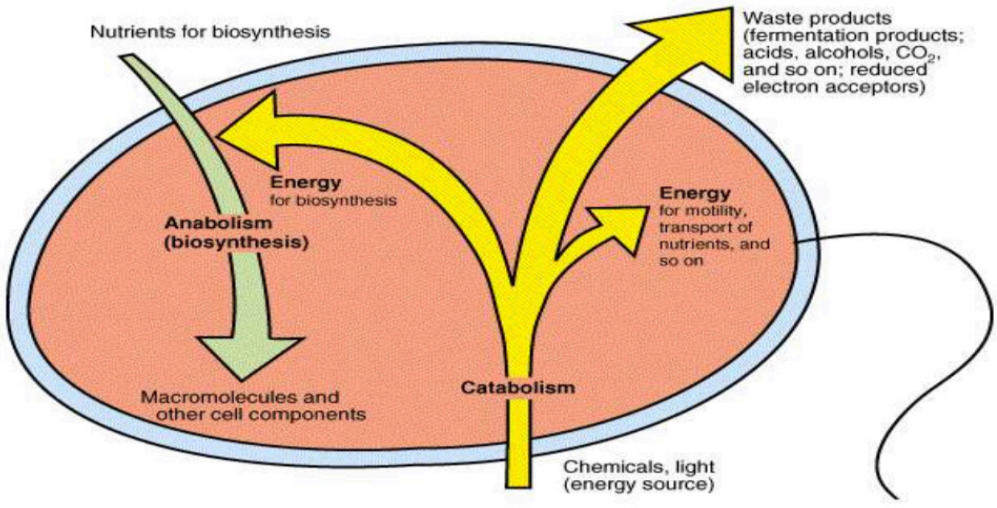
As mentioned before :

• Metabolism = Anabolism + Catabolism.

• Anabolism = synthesis.

•Catabolism = degradation.

• Understanding physiology & metabolism is necessary for bacterial identification & to design antibacterial agent



-Nutritional requirements:

• Nutritional requirements differ among bacteria and can be used for identification

1-Many organic and inorganic elements are present in bacteria, even for structural or for functional.

A. carbon, hydrogen, Oxygen, nitrogen, phosphorus and sulphur: needed for the synthesis of structural components.

B. potassium, calcium, magnesium and iron: needed for cellular functions.

2-Can be obtained from simple elements or by breaking down large molecules such as protein breakdown into amino acids using bacterial enzymes

3-Many bacteria have to synthesize some nutrients such as folic acid which makes these bacteria susceptible to agents that interfere with the biosynthesis of folic acid, e.g by *trimethoprim* & sulfonamides antibiotics. So these are used to interfere with the synthesize of bacterial DNA, thus prevention

Bacteria use **folic acid** in order to synthesize the nucleic **acids** that make up their DNA.

• Nutrients can be obtained from different sources

1-Elements such as:

A. hydrogen & oxygen are obtained from water.

B. Carbon: usually obtained from degradation of carbohydrates by oxidation or fermentation.Carbon is necessary to provide energy in the form of ATP (adenosine triphosphate).

C. Nitrogen: from ammonia in the environment or proteins ' by deamination' using bacterial enzymes

2. Organic factors (from exogenous source/can't be synthesized by bacteria) such as: Amino acids: e.g from proteins breakdown. Purines and pyrimidines:

* Nucleic acid precursors.

* must be converted into nucleotides(sugar+base+phosphate) & nucleosides(sugar+base) before being incorporated into the DNA or RNA.

3. Vitamins: most are needed for the formation of coenzymes in some bacteria.

Major elements sources

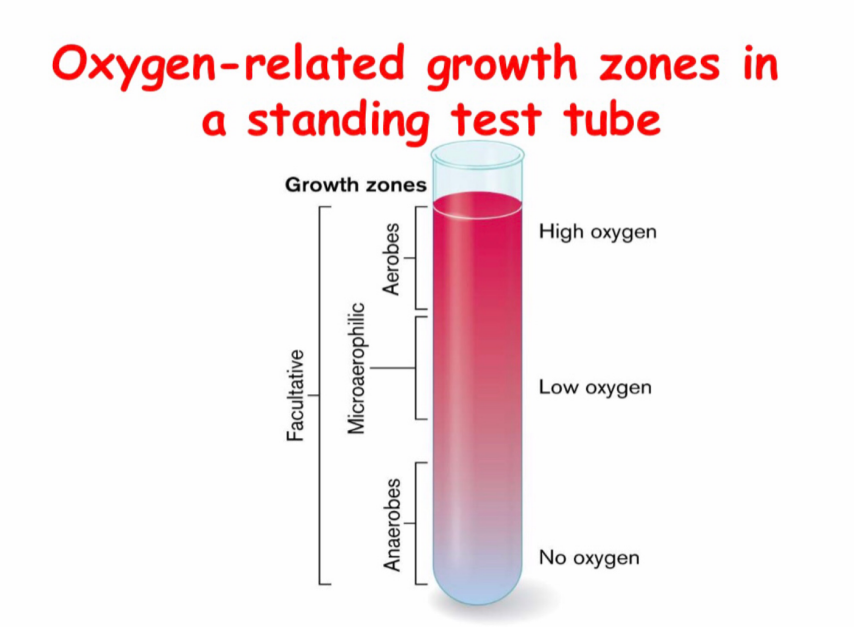
Needed for Structural Components

Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, So, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Needed for Cellular Function

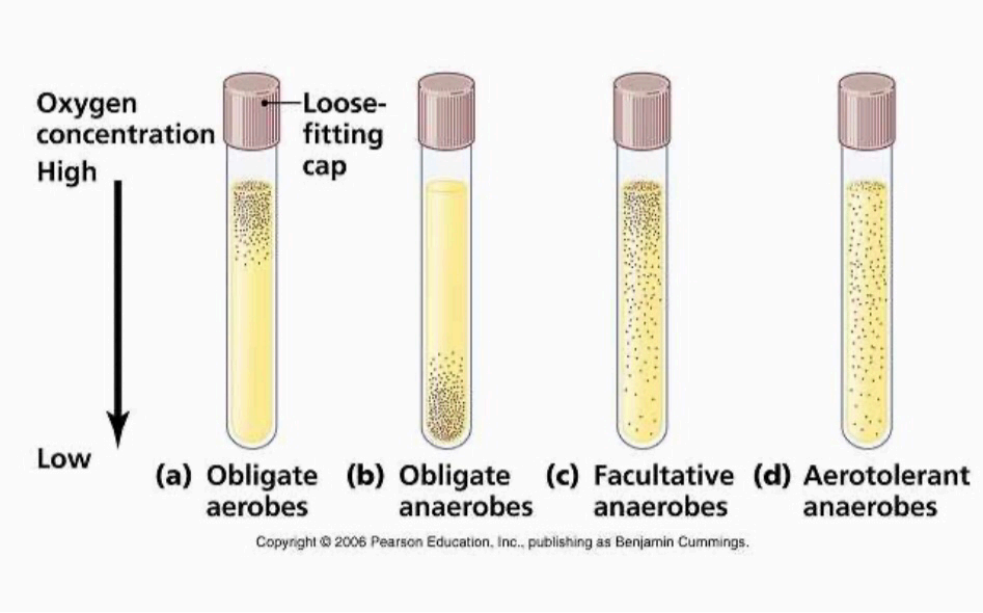
-Source and functions of elements are required.



The concentration of oxygen on the top of the test tube is high and in the bottom there is no oxygen, so when ever we go downward to the bottom, the oxygen concentration is getting less. So if we recognize that turbidity is some where in the tube , then we conclude that this kind of bacteria prefer high/low/no oxygen.

-The agar helps retard oxygen diffusion and helps maintain the stratification of organisms growing in different layers of the broth..

-Obligate aerobes will only grow in this oxygen-rich top layer.
-Obligate anaerobes will only grow in the lower areas of the tube.
#Note: usually when a clear liquid become turbid, all over the liquid becomes turbid, not discrete as we mentioned earlier. And I asked the doctor about that, she said that the whole liquid becomes turbid.



-We can see here that obligate aerobes prefer a higher concentration of O_2.
-Aerotolerant anaerobes can bear and tolerate O2 rich top, and so on.

Table 4.1 Key descriptive terms used to categorize bacteria according to their growth requirements

Descriptive term	Property	Example
Growth atmosphere		
Strict (obligate) aerobe	Requires atmospheric oxygen for growth	*Pseudomonas aeruginosa*
Strict (obligate) anaerobe	Will not tolerate oxygen	*Bacteroides fragilis*
Facultative anaerobe	Grows best aerobically, but can grow anaerobically	*Staphylococcus* spp., *E. coli*, etc.
Aerotolerant anaerobe	Anaerobic, but tolerates exposure to oxygen	*Clostridium perfringens*
Micro-aerophilic organism	Requires or prefers reduced oxygen levels	*Campylobacter* spp., *Helicobacter* spp.
Capnophilic organism	Requires or prefers increased carbon dioxide levels	*Neisseria* spp.
Growth temperature		
Psychrophile	Grows best at low temperature (e.g. <10°C)	*Flavobacterium* spp.
Thermophile	Grows best at high temperature (e.g. >60°C)	*Bacillus stearothermophilus*[a]
Mesophile	Grows best between 20–40°C	Most bacterial pathogens

[a] Not a pathogen; its spores are very heat resistant and are used for testing the efficiency of heat sterilization.

To summarize what we have taken (Bacterial physiology , growth and metabolism).

• Bacterial growth is an active mechanism.

• Bacteria has different nutritional needs and nutritional uptake mechanisms.

• Nutrients are metabolized using many bacterial pathways.

• Growth has many phases and it is affected by the surrounding environment e.g oxygen & temperature.

• Bacteria has to replicate its DNA in order to pass it to the offspring.

Thanks ALLAH