6

Microbiology

Doctor 2018 | Medicine | JU

Sheet

Slides

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Contributed in the Scientific Correction

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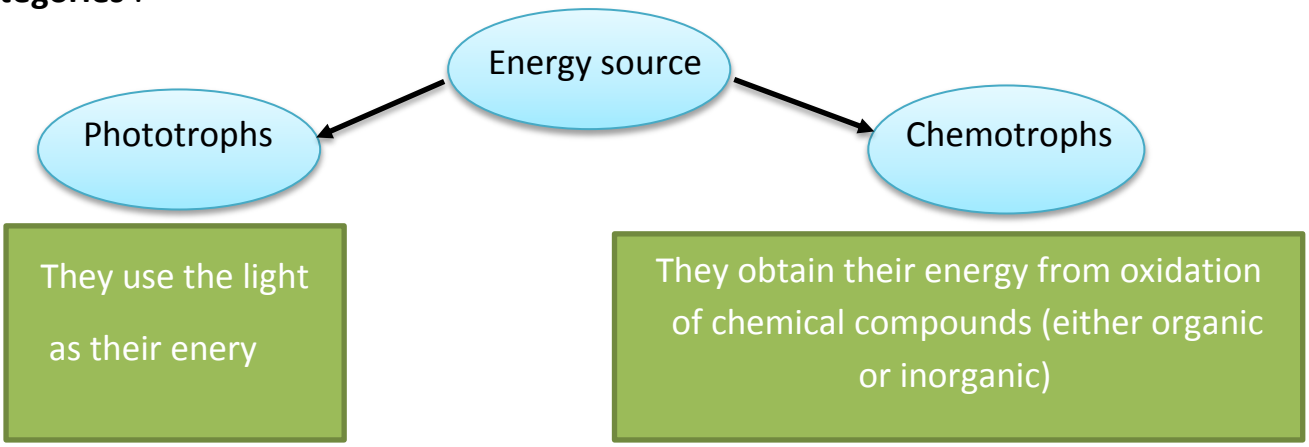
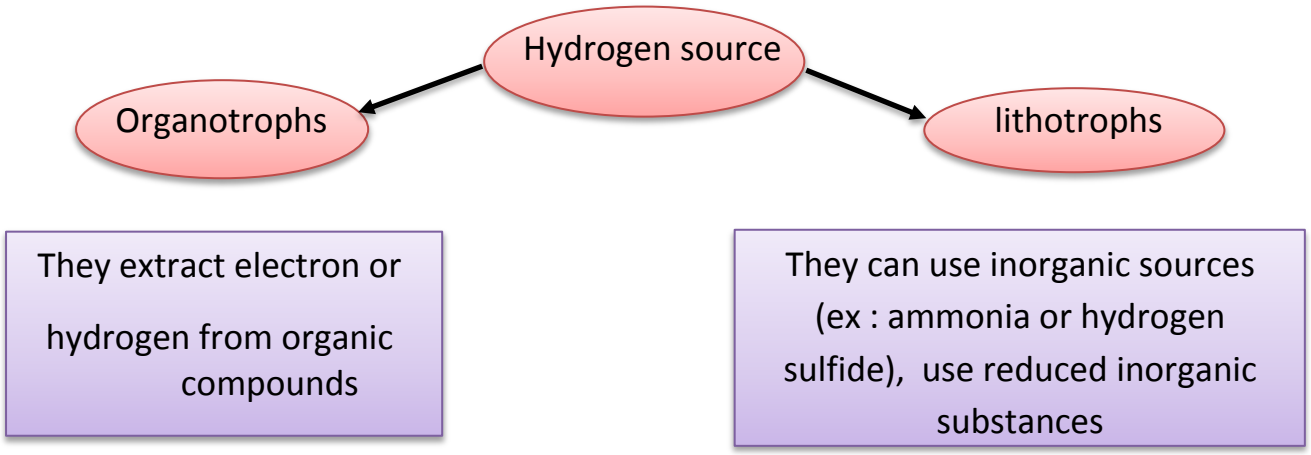
Doctor

Ala’a

According to how bacteria takes energy or hydrogen or carbon , we put them in categories :

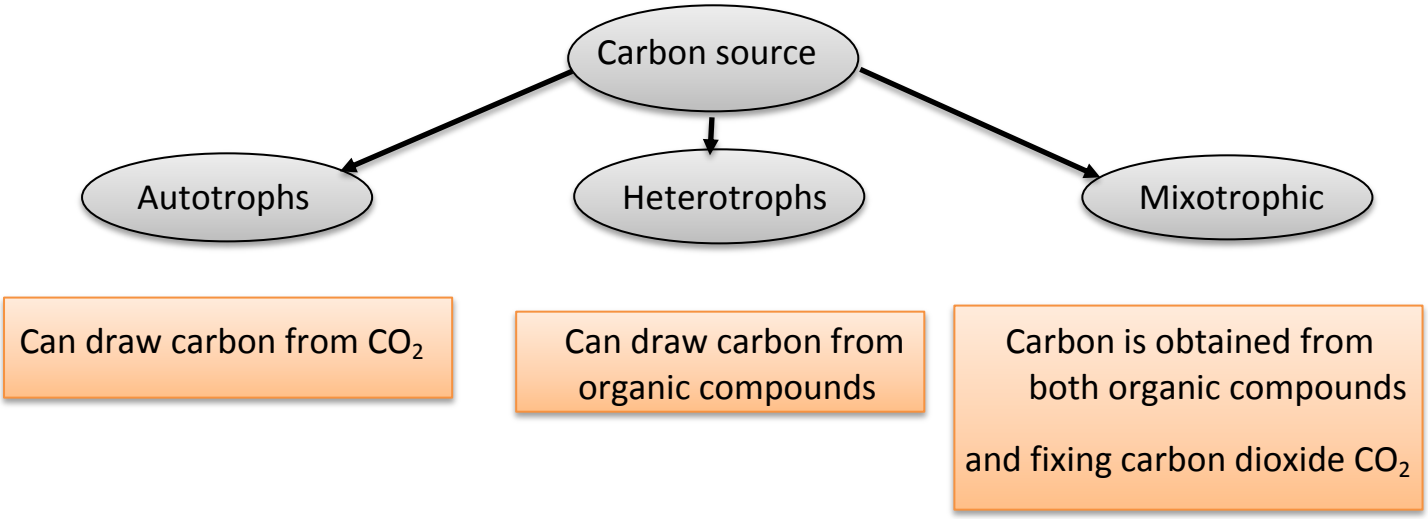




• Energy and Hydrogen donor designations are referred to routinely by combining the two terms :

- **Chemo-organotrophs :** (chemo) that's mean it obtained energy from oxidation chemical compounds , and (organo) means it extract hydrogen from organic compounds .
  (The vast majority of currently recognized medically important organisms) .

- **Chemo-lithotrophs :** It obtained energy from oxidation chemical compounds and use inorganic sources .
  Example : Some Pseudomonas species .



These requirements can be combined (Energy + Carbon) sometimes :

- **Chemo-heterotrophs :** They obtained energy from chemical compounds , carbon from organic compounds .
  This group includes most of microorganisms as well as all protozoa , fungi and animals .

Inside the cell , sugar molecules or other sources of carbon and energy are metabolized by different pathways , mainly by :

1) The Embden–Meyerhof glycolytic pathway .

2) The pentose phosphate pathway .

3) The Krebs cycle to yield the carbon compounds needed for biosynthesis .

Note : Dr.Alaa said that we should know them in general and not to go through details .

Bacteria generates energy by two ways : fermentation , and oxidation .

Comparison of metabolism :

1) Aerobic respiration (oxidation) : the total ATP produce in prokaryotes = 38 , and in eukaryotes = 34 .

- The final electron receptor is usually O_2 .

2) Fermentation : the total ATP produce = 2 !! "Less efficient" .

- The final electron receptor is organic molecule .

- The end products of this way is : Acids / Alcohol .

Note : CO_2 is produced in both .

Environmental conditions governing growth :

1) Temperature : majority of medically important bacteria are mesophiles "grow at 37°C" (body temperature) .

2) Water : at least 80% of bacterial cell consists of water .

3) Oxygen and carbon dioxide .

In bacteria that's do aerobic respiration , it must have protective enzymes because this metabolism may give rise some toxic substances , like Hydrogen peroxide (H_2O_2) and superoxide anion (O^{-2}) .

Superoxide is partially detoxified by an enzyme called superoxide dismutase .

Hydrogen peroxide is degraded by peroxidases (Catalase) .

Bacteria that possess these protective enzymes can grow in the presence of oxygen.

* In response to oxygen, bacteria can be placed in five categories as in the next Figure :

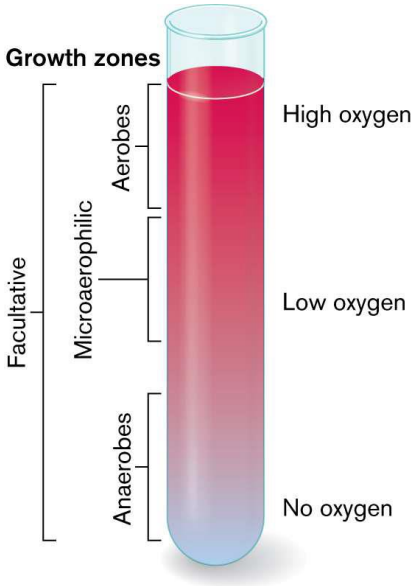
Table 4.1 Key descriptive terms used to categorize bacteria according to their growth requirements

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.

Note : this table is for memorizing , the doctor said that you can memorize it now or later when we take each type of bacteria in details .

Oxygen-related growth zones in a standing test tube :

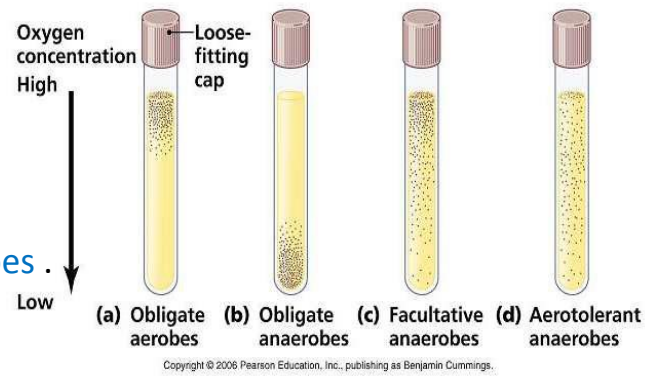


1) At the top of test tube , usually we have **High** level of oxygen so we call them Aerobes .

2) At the bottom of test tube , there is **NO** oxygen so we call them Anaerobes .

3) At the middle of test tube , there is **Low** level of oxygen so we call them Microaerophilic .

This figure represent the distribution of bacteria when we have certain concentration of Oxygen .



a) They need oxygen , so they are aerobes .

b) They don't need oxygen , so they are anaerobes .

c) They grow best aerobically , but also grow anaerobically , so they are facultative anaerobes .

d) They are anaerobes , but tolerate exposure to oxygen , so they are aerotolerant anaerobes .

Distribution of bacteria according to the pH that live in :

1) Majority of bacteria grow **BEST** at neutral or slightly alkaline pH (it is around 7 – 7.4) .

2) Acidophiles **:** grow **BEST** at low pH (it is around 0 – 1) .

Example : ThioBacillus (pH = 6.5 – 6.8) .

3) Alkalophiles **:** grow **BEST** at high pH (it is around 10) .

Example : Vibrio Cholerae (pH = 8.4 – 9.2) .

Bacterial physiology and metabolism Summary :

1) Bacterial growth is an active mechanism .

2) Bacteria has different nutritional needs and nutritional uptake mechanisms .

3) Nutrients are metabolized using many bacterial pathways .

4) Growth has many phases and it is affected by the surrounding environment , like O_2 and temperature .

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

NOW let us talk about identification and classification of bacteria …

Firstly , we will start with identification of bacteria :

The successful identification of microbiological agent depends on :

1- Proper aseptic techniques .

2- Correctly obtaining the specimen .

3- Correctly handling the specimen .

4- Quickly transporting the specimen to the lab .

5- Once reaches the lab it is cultured and identified .

* After the microbe is identified , it is used in susceptibility tests to find out the effective control measure .

What does susceptibility(sensitivity) tests mean ?

We see kinds of antibiotics that we use on different patients .

lets take an example : we have two patients , every one of them has Pharyngitis , lets say the same bacteria in both , but the response of the antibiotics for this bacteria in first patient is different than the second one .

The methods use to identify bacteria fall into three categories :

1) Phenotypic "morphology" (it could be Macroscopic and Microscopic)

Microscopy (staining)

Growth on enrichment , selective , differential media specimen biochemical test (rapid test methods) " also we will talke about them in details" .

2) Immunological (serological) tests .

3) Genotypic (Molecular techniques) .

Phenotypic Methods :

A) Microscopic morphology :

Include a combination of cell shape , size , Gram stain , acid fast , also special structures like endospores , granule and capsule , those can be used to give an **initial putative identification** .

Usually done by using :

- ✓ Simple stain
- ✓ Gram stain
- ✓ Acid – fast stain (Ziehl – Neelsen stain) " used in cases of mycobacterium tuberculosis"
- ✓ Special stains

B) Macroscopic morphology :

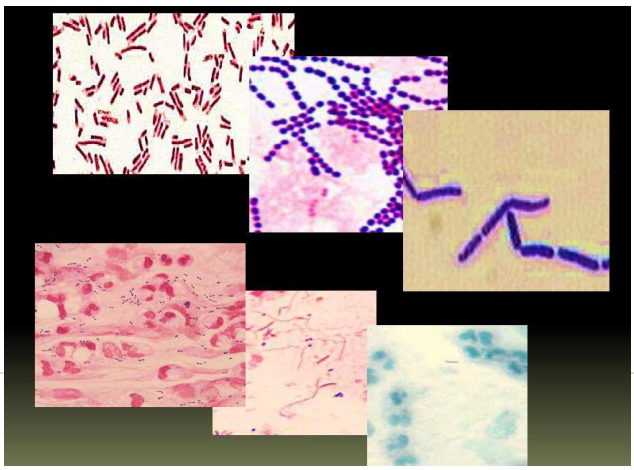
Bacterial cultivation (isolation of bacteria from the specimens of patients) .

Principle of cultivation :

- Nutritional requirements .
  - non – fastidious : simple requirements for growth .
  - fastidious : complex , unusual , or unique requirements for growth .
- Streaking for isolation .
- Streaking for quantitation .

These are some different stains we use it

to help us in identification of bacteria .

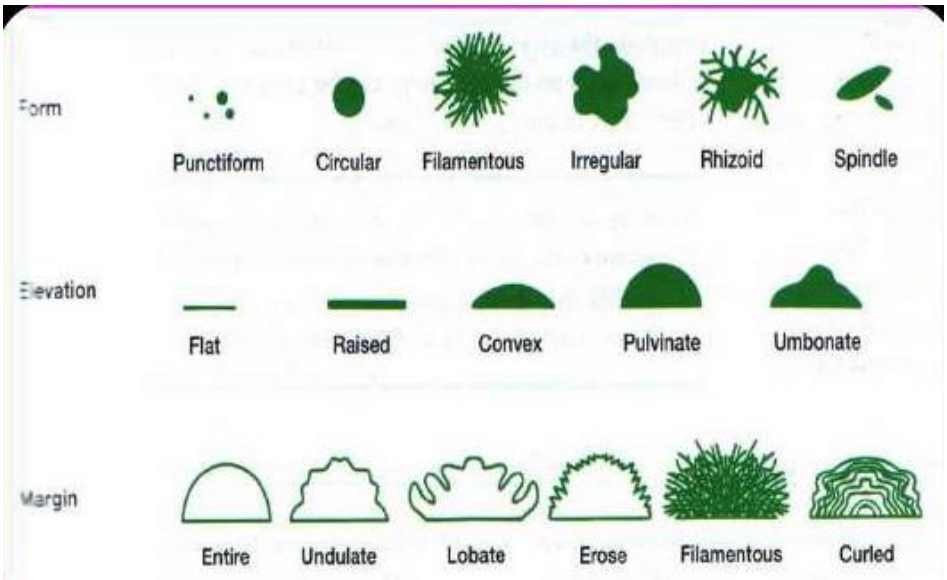


Note : in slide 7 , we have already talked about the method of streake in sheet 2, so there is no need to repeat ☺<3

REMEMBER: a pure culture mean that we have a single type of bacteria .

Colony characteristics : with the naked eye , e.g. : texture , shape , pigment , growth pattern .

- Colony form : pinpoint , circular , filamentous , irregular .
- Colony elevation : flat , raised , convex .
- Colony margin : smooth , irregular .



Types of culture media :

1) Basal media : used for culture of bacteria that don't need enrichment of the media .

Examples : Nutrient broth , nutrient agar and peptone water

This bacteria is **non – fastidious** .

2) Enriched media : by adding blood , serum or egg .

Examples **: blood agar , chocolate agar** " blood that heat it to certain temperature , bring out some factors from RBCs and the color of media is converted from red to dark brown " , and **Lowenstein-Jensen media** .

chocolate agar blood agar

3) Selective media : contains agents that inhibit the growth of all agents except that being sought (dyes , bile salts , alcohols , acids , antibiotics) .

Why also antibiotics ?

For example , because I want to kill all gram positive bacteria , because I looking for gram negative bacteria .

Example : Salmonella Shigella Agar (SSA) , Mannitol Salt Agar .

4) Differential media : This media is **capable to differentiate between species** .

An indicator is included in the medium .

A particular organism causes change in the indicator , e.g. blood , neutral red

Examples : blood agar and MacConkey agar .

Lets take about this type of media knowing that we will study all the details later on :

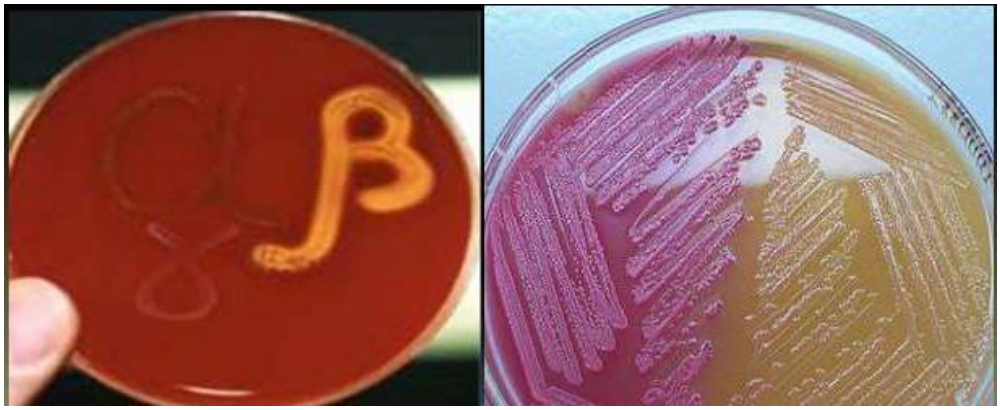
Blood agar is a differential medium that distinguishes bacterial species by their ability to break down the red blood cells included in the media. Blood agar is often used to distinguish between the different species of pathogenic Streptococcus bacteria. The different types of Strep each have a predictable pattern of hemolysis, which is simply the breakdown of red blood cells .

We have three type of hemolytic streptococcus : alpha , beta and gamma .

Some of this bacteria make the beta hemolysis because it has some enzymes and it makes a clear zone in colony .

Alpha hemolytic make discoloration around the colony during the partial breakdown of the RBCs.

Gamma don't chang the media appearance (non hemolytic) so we will see the colony without any hemolysis



5) Transport media : these media are used when specimen can not be cultured soon after collection .

Examples : Cary-Blair medium , Amies medium , Stuart medium .

6) Storage media : used for storing the bacteria for long period of time .

Biochemical tests :

The microbe is cultured in a media with a special substrate and tested for an end product .

Prominent biochemical tests include enzymes (catalase , oxidase , decarboxylase) , fermentation of sugars , acid or gas production and hydrolysis of gelatin .

Those mainly that's we work on it .

Other biochemical tests of interest include :

1) Indole test .

2) Methyl Red / Vogues – Proskauer .

3) Citrate utilization .

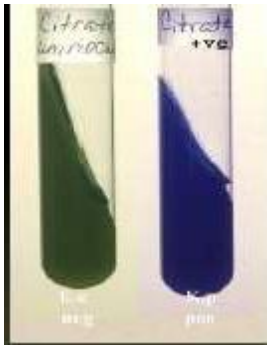
4) Coagulase test .

5) H_2S Production (TSA) .

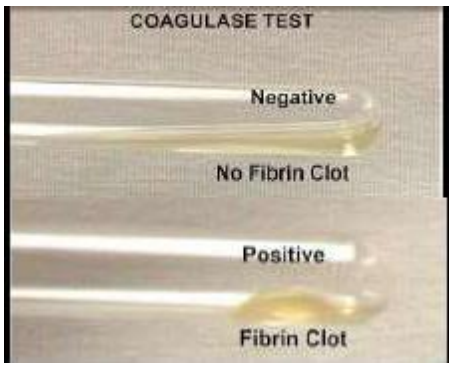
6) Urease test .

7) Phenylalanine deaminase test .

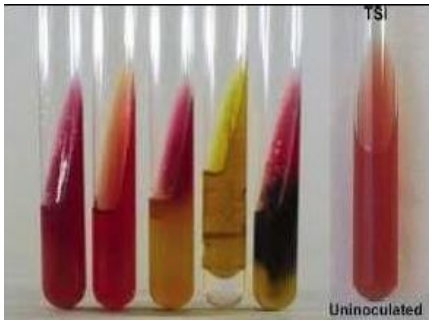
Indole test



Citrate utilization

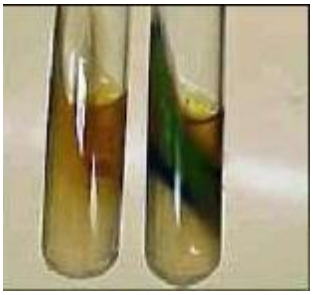


Coagulase test



H_2S Production (TSI)

Urease test



Phenylalanine deaminase test

☺Good luck☺

Don't hesitate to ask us !! ;)