



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
الَّذِي عَلَّمَ بِالْقَلَمِ ۖ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ



MICROBIOLOGY

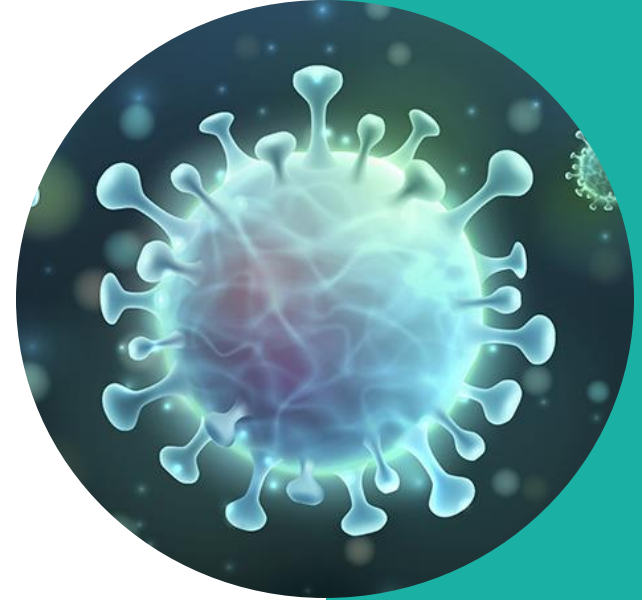
MID | Lecture 4

Bacterial Growth & Physiology

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Color coding used in the modified:



Black: the original slides



Teal: the doctor's explanation/words



*Gray: additional information and
explanation*

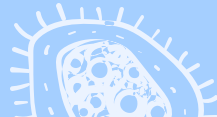


Red: important information



Lecture 3

Bacterial Growth & physiology



Bacterial Growth & Physiology

Definition of Bacterial growth

Binary Fission

culture media

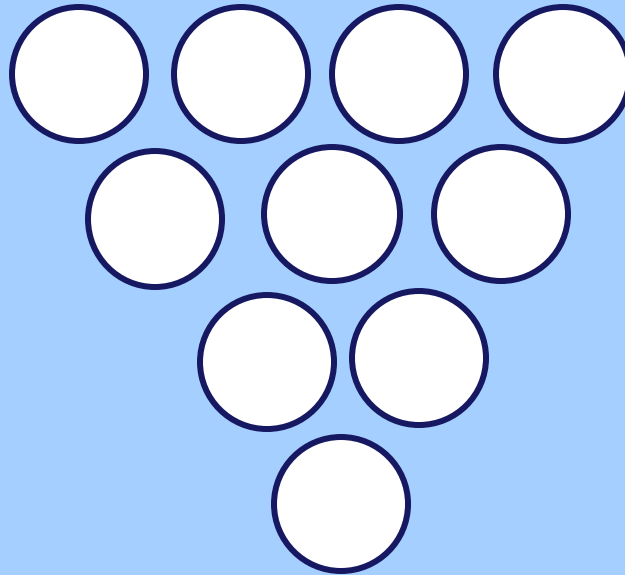
- A) Definition
- B) Classification
- C) Types

Bacterial growth curve

Bacterial growth requirements

Bacterial Growth: Definition

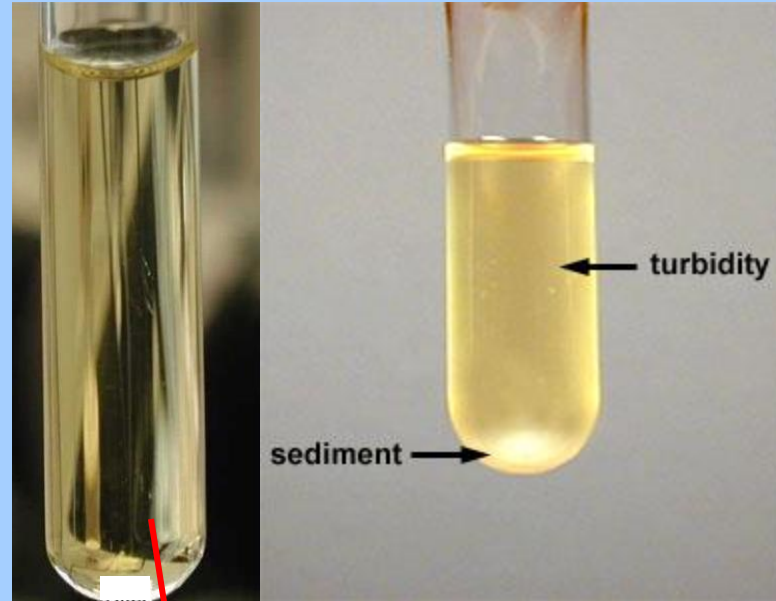
**Size
& Number of
organism**



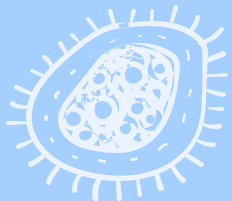
1) Bacterial Growth

Indicated by

a) Turbidity of
the fluid media



The liquid is called broth

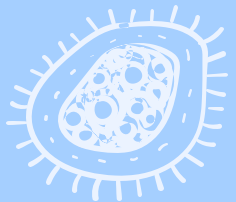


1) Bacterial Growth

Indicated by

b) Colonies on solid media

(Macroscopic product)



Detection of Bacterial Growth:

There are two methods to detect whether bacterial growth has occurred or not: **1.Detection by**

Turbidity in Broth : If we take a sample and use a liquid culture medium, this liquid medium is called (broth).After inoculating the broth with the sample and leaving it for several hours, we may observe (turbidity), which means cloudiness of the liquid.

=>**Clear broth**: If there are no bacteria growing in the medium, the broth remains clear.

=>**Turbid broth**: Once turbidity develops, it indicates that bacterial growth has occurred.

For example, the culture medium shown in the figure is called (broth). Initially, it is clear. However, after bacterial growth occurs, the medium becomes cloudy (turbidity)due to the presence of bacteria.

2. Detection of Bacterial Growth on Solid Media :Another method of detecting bacterial growth is by observing colonies on solid media.

When bacteria grow on solid culture media, they form visible clusters called colonies.These colonies can be seen with the naked eye; therefore, they are called macroscopic colonies.

Colonies may appear as small circular structures or sometimes as tiny dots (points).

A Summary:Bacterial growth can be detected by observing turbidity in liquid media (broth) or colonies on solid media

2) Colony (Macroscopic product)

Single bacterium

On solid media

Binary fission

After 20-30 generations

Colony: around 1 million



How do we obtain a bacterial colony?

A bacterial colony is obtained by inoculating a single bacterium onto a solid culture medium. The solid culture medium is poured into a **Petri dish** (a laboratory dish used for culturing microorganisms).

The bacteria are inoculated onto the solid medium, the bacterium begins to reproduce through binary fission (a method of bacterial cell division).

Binary fission is the process by which one bacterial cell divides into two daughter cells.

One parent bacterium divides into two daughter cells. The two daughter cells divide to produce four cells. The four cells divide to produce eight cells, and the process continues exponentially.

After approximately 20-30 generations, a visible bacterial colony may develop. Each colony may contain approximately one million or more bacterial cells

The number of bacterial cells increases according to the following formula:

$$N=2^n$$

N: number of cells

n: number of cell divisions

(2) is the base because each binary fission gives two daughter bacteria cells

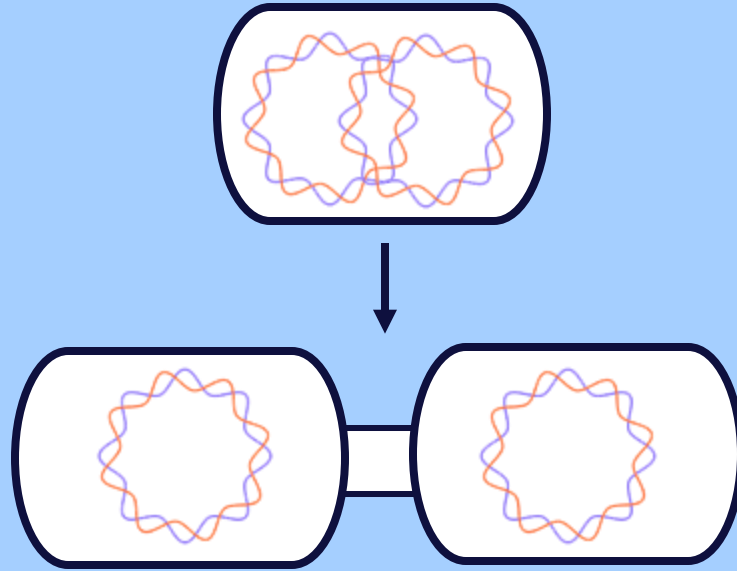
3) Generation time (doubling time)

The **generation time**, also called the **doubling time**, varies from one bacterial species to another. It refers to the **time required for a bacterial population to double in number under specific growth conditions**.

Some bacteria have a very short generation time and can divide approximately every 13 minutes. Other bacteria have a much longer generation time and may require approximately 24 hours to complete a generation.

13min (*V.cholerae*)

Vibrio cholerae



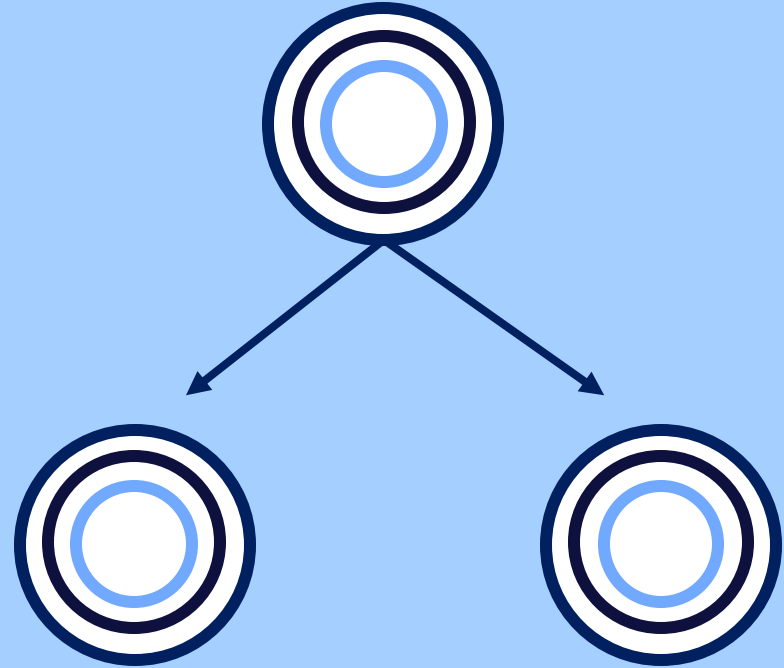
24 hrs (*M.tuberculosis*)

Mycobacterium tuberculosis

Binary fission

- Concept of Binary Fission

It is a process where a single parent cell divides to produce two identical daughter cells.

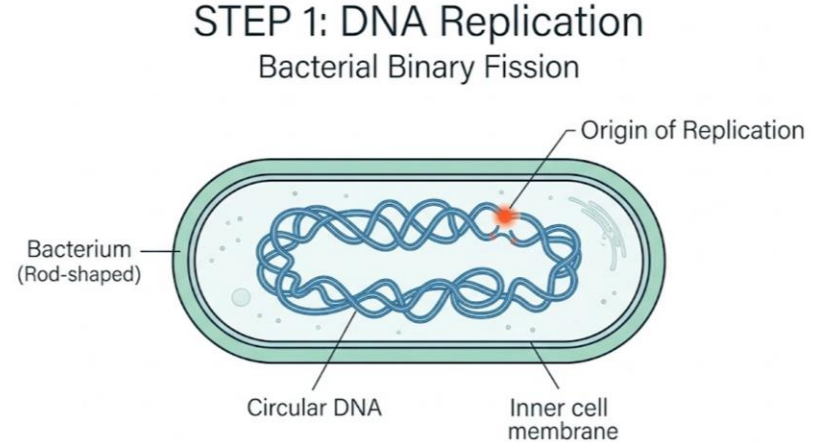


Binary Fission

Step 1: DNA Replication

The circular, double-stranded DNA (dsDNA) replicates.

Two identical copies of dsDNA are formed.

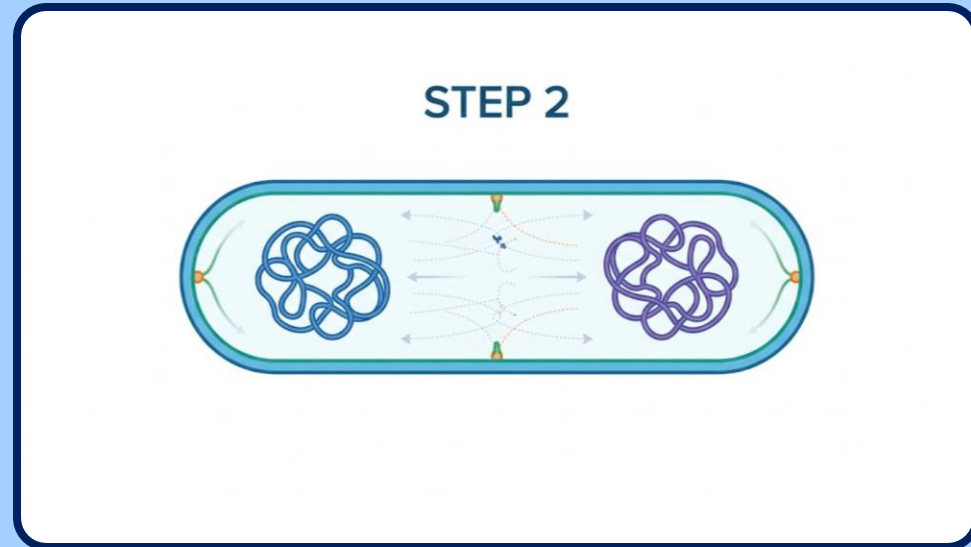


Binary Fission

Step 2: Cell Elongation

The bacterial cell elongates (grows in length).

The two identical dsDNA copies move toward opposite poles.

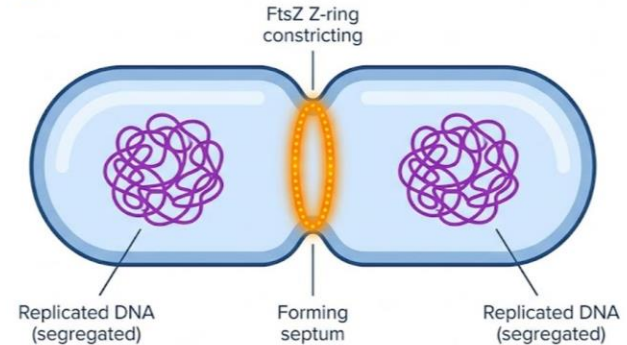


Binary Fission

Step 3: Septum Formation

A division septum begins to form in the center of the cell.

STEP 3



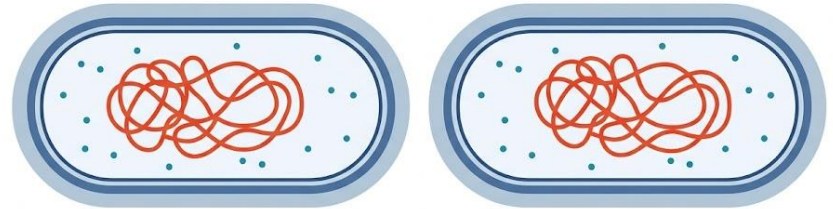
Binary Fission

Step 4: Cell Separation

The cell wall pinches completely through the center.

The cell splits into two identical independent daughter cells.

Step 4: Two Identical Daughter Cells



The parent cell has completely split, resulting in two identical, independent daughter cells, each with its own complete copy of the circular DNA.

Bacterial culture media

Bacteria grow
(In vitro)



Need nutrients for
growth

Culture media are supplied with nutrients required for bacterial growth , including , proteins , sugars , salts

Culture media : nutrient-containing artificial media used to grow bacteria in vitro (outside the human body)



Culture refers to the process of growing microorganisms in a culture media under controlled conditions

Artificial

Purpose of bacterial culture

1

Detection of bacterial growth

Culture media using Petri dish



To determine if there is bacteria or not , is the infection caused by bacteria or not
For example , if we culture a sample , using the appropriate culture medium and correct procedures , and no bacterial growth occurs that means it's not a bacterial infection.
It could be a viral infection , but **virus don't grow on cultures media , it needs a living cell.**

Purpose of bacterial culture

2

Isolation & Identification of the Causative agent



After we isolate bacterium, we perform **biochemical tests** to identify it

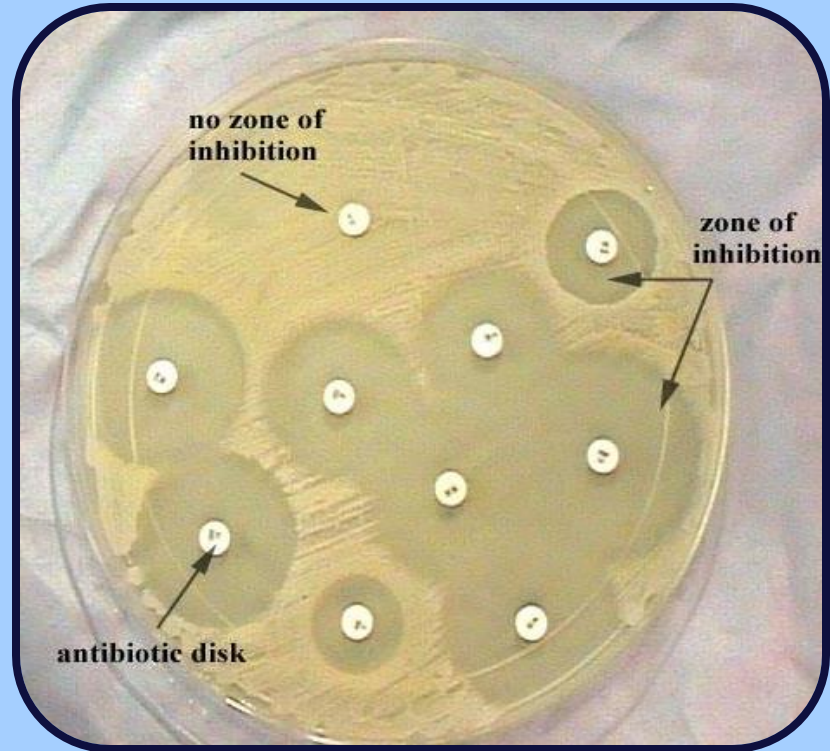
Why do we need to identify it ?

To determine **which causative agent** is responsible for causing the disease

Purpose of bacterial culture

3

Antibiotic Susceptibility Testing (AST)



Antibiotic Susceptibility Test (AST):

After identifying the causative agent, we need to perform an antibiotic susceptibility test (AST). The (AST) helps us **determine which antibiotics can be used to treat the patient and which ones the bacterium is sensitive to, meaning they are effective against it.**

The (AST) is not the same for every patient .

For example, patient X may have *Staphylococcus aureus*, and patient Y may also have *Staphylococcus aureus*. Although both patients have the same bacterial species, we still need to determine which antibiotic is appropriate for each patient.

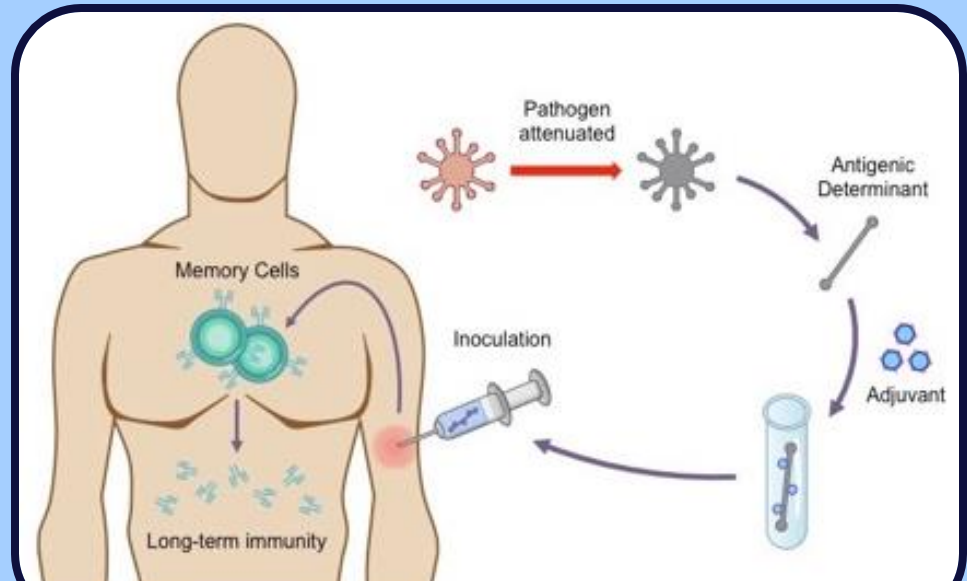
For example, the bacterial strain in patient X may be **methicillin-resistant** *Staphylococcus aureus* (MRSA), meaning it is **resistant to methicillin**. The bacterial strain in patient Y may not be resistant to methicillin.

Therefore, we must perform an (AST) to determine the appropriate antibiotic for each patient.

Purpose of bacterial culture

4

Preparation of vaccines & Other biological products



An example of biological products, a bacterium called (***Streptococcus pyogenes***) produces an **enzyme** called (**streptokinase**). We can obtain the enzyme (streptokinase) from (*Streptococcus pyogenes*) and use it to **treat patients who develop blood clots**.

Classification of media

Liquid



Liquid in a **test tube** ,
it's called **broth**

Solid



Transparent plate and has a lid ,called Petri dish (named after the scientist who made it) ,
we pour the solid media in them , we often use **agar** here,

Types of media

Those are the frequently used media

1) Simple media

2) Enriched media

3) Selective media

4) Differential media

It may be (differential and selective) medium ,
or just differential medium



Simple media

**Basic requirement for
growth of most bacteria**

It provides the **minimum nutrients** required
for the bacteria



Simple media

Examples of simple media

A) Peptone water

What does it contain ?

Peptone + 0.5% NaCl

What's its purpose or use ?

- Enhances bacterial growth**
- Used as a base for Sugar media**



Simple media

B) Nutrient broth

What does it contain ?

Peptone + Meat extract

What's its purpose or use ?

Enhances bacterial growth



Simple media

C) Nutrient agar plate

What does it contain ?

Nutrient broth + 2%

agar agar

(Seaweed)

Agar is extracted from seaweed and gives solidity to the media , after we mix the broth with agar , we boil them together and then freeze them , after that we pour them to the Petri dish and it becomes a jelly-like texture



Staph. aureus

Enriched media

Fastidious bacteria
Need blood, serum for
growth



Fastidious bacteria (المطلبة أو المتطلبة) : bacteria that needs blood, serum (blood plasma without clotting factors), it needs specific proteins ,etc..

Enriched media

A) Blood agar

What does it contain ?

- Nutrient agar (cooled to 45-50 °C)
- sheep blood (5-10 %)

From the simple media

The RBCs in the blood gives a lot of factors that nourishes the bacteria

Ex: *Streptococcus spp.*

Streptococcus species

After boiling the nutrient agar , we let it cool a bit and then we add sheep blood



Hemolysis on blood agar:

Some bacteria have the ability to hemolyze the RBCs from the sheep blood in the blood agar, which helps in identification of bacteria

Complete hemolysis of RBCs, it makes a clear zone

A. Complete (beta) hemolysis:

- *Staphylococcus aureus*
- *Streptococcus pyogenes*

C. No (gamma) hemolysis:

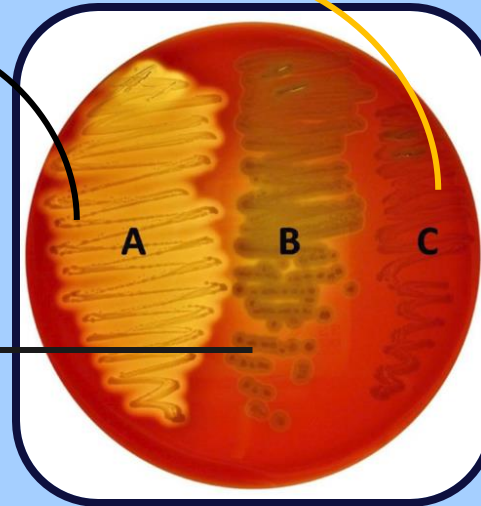
- *Enterococci.*

None hemolytic

Partial hemolysis, usually Greenish zone

B. Partial (alpha) hemolysis:

- *Streptococcus viridans*
- *pneumococci.*



Enriched media

B) Chocolate agar

What does it contain ?

- Nutrient agar + Blood (heated to 80 °C)
- Lysed RBCs release Haematin (X & V factors)
- Gives the chocolate –brown color

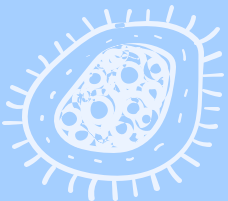
We boil the nutrient agar and blood to 80 Celsius, then the RBCs lysis occurs producing 2 important factors , X and V factors giving the brown color

Hb

Heat

Haematin (Chocolate)

Haematin contains X and V factors



Those bacteria only grows in this media because they need those factors to grow . They can be called **fastidious**

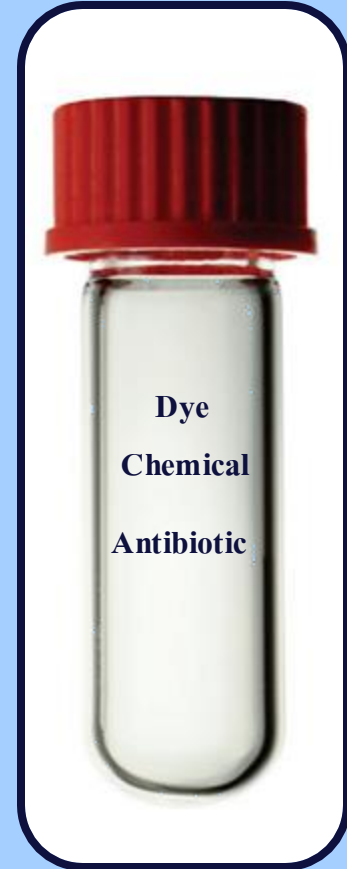
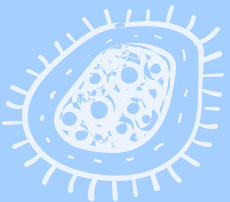
Haemophilus spp.

Neisseria spp.

Selective media

What is it's features?

- **Allows the growth of specific organisms**
- **Inhibits the growth of unwanted organisms**
- **Isolation & selection**



Selective media allow the **growth of a specific organism** while **inhibiting the growth of unwanted organisms**.

Selective media are used to grow the bacteria we are looking for while inhibiting the growth of other bacteria.

Several factors help us determine which bacteria we are looking for, including the **source of the specimen**, the **patient's age**, and the **disease the patient has**.
These factors help us **predict** whether the suspected bacteria are **Gram-positive or Gram-negative**.

For example, if I suspect that the **bacteria are Gram-negative**, I can use a culture medium that **inhibits the growth of Gram-positive bacteria**.
And also it may be used to inhibit the growth of normal flora bacteria

This medium is called selective media because it allows the growth of certain bacteria while inhibiting the growth of other bacteria that we are not looking for.
These factors help us with the processes of isolation and selection of the desired bacteria.

How is the selective medium “selective “ ?
Selective media contains substances that inhibit the growth of unwanted bacteria, such as , a **specific dye , chemical, or antibiotic**

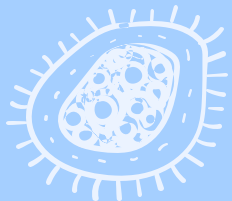
Selective media

Examples for selective media :

Lowenstein Jensen medium

Malachite green

Mycobacterium tuberculosis



Lowenstein-Jensen medium Is used to isolate and grow mycobacterium tuberculosis, it contains malachite green , which inhibits any contaminating bacteria except mycobacterium tuberculosis (suppresses the growth of other bacteria)



Selective media

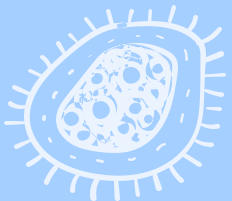
- **Blood tellurite agar**
- **Contains Potassium Tellurite**
- **Selective for: *C. diphtheriae***

We use it when we want to search for *Corynebacterium diphtheriae*

What's its feature?

(Produces characteristic black colonies)

It's named Blood tellurite agar because it contains potassium tellurite



Differential media

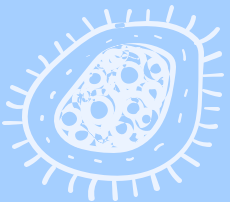
Differential &
selective media

- **Contains indicators (like dyes or sugars)**

Why do we use it ?

- **Differentiates between look-alike microbes**
- **Causes a (visible change) in colonies or medium**
- **Some differential media are also considered as selective media**

The change could
be in color



MacConkey's agar

It is a useful media if u r looking for G-ve bacteria

Selective agents: Bile salts & Crystal violet (Inhibit Gram-positive)

Differential agents: lactose

(Sugar) + Neutral red (pH Indicator)

Lactose is to differentiate between fermenter and non fermenter bacteria

Results:

Pink colonies = Lactose Fermenters

Pale colonies = Non- Lactose Fermenters

Crystal Violet here isn't for staining but as selectivity factor to inhibit G-ve bacteria



Pink

Pale

Mannitol salt Agar (MSA)

Selective agents: 7.5% NaCl (Inhibits non-Staphylococcal organisms)

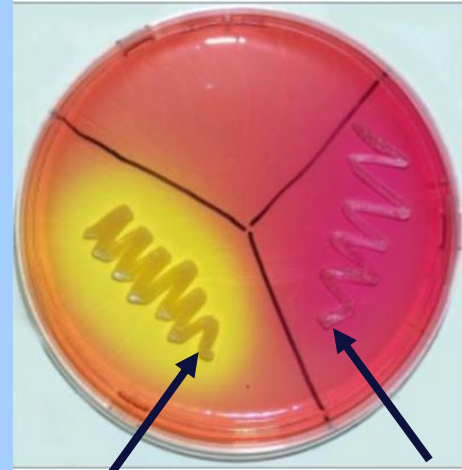
Differential agents: Mannitol + Phenol red (pH Indicator)

Results:

***Staphylococcus aureus* : Ferments Mannitol**
(Yellow colonies /medium)

***Staphylococcus epidermidis*: Non-Mannitol Fermenter**
(Pink colonies/medium)

There r many types of satphylococcus bacteria including aureus, epidermidis, hemolyticus.... Using Mannitol salt agar helps u identify the types that ferments mannitol



*Staphylococcus
aureus*

Staphylococcus epidermidis

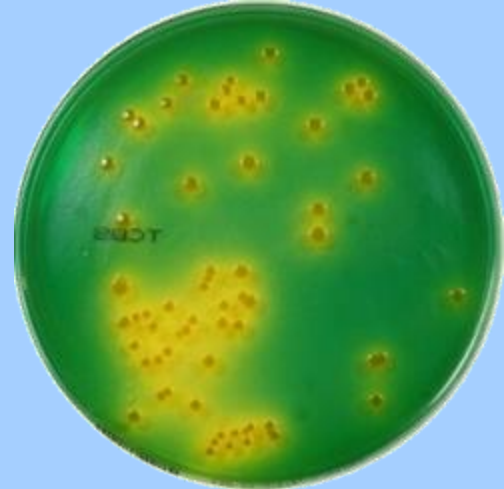
Thiosulfate-Citrate-Bile-Sucrose Agar. (TCBS)

Selective agents: Thiosulfate, Citrate, Bile salts
(Inhibit Gram-Positive & Enterobacteriaceae)

Differential agents: Sucrose, Bromothymol blue
(pH Indicator)

Selective for: *Vibrio* spp. (e.g: *Vibrio cholerae*)

Usually used to identify *vibrio cholera* , which means it's G-ve bacteria



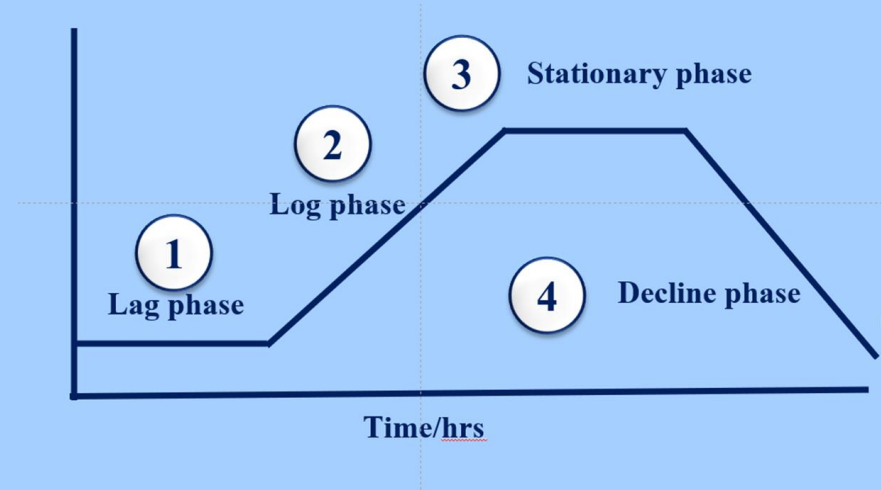
Bacterial growth curve

- 1) Lag phase
- 2) Log/exponential phase
- 3) Stationary phase
- 4) Decline phase

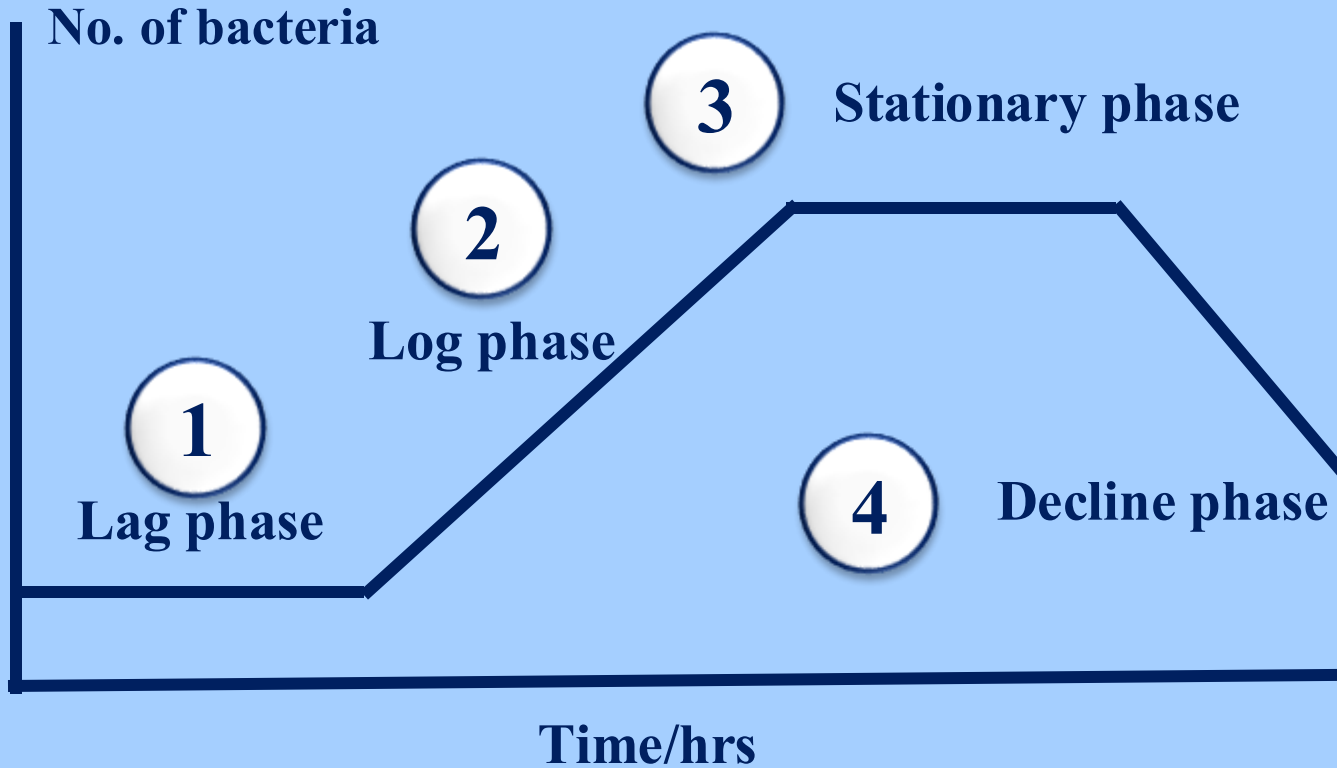
When we grow bacteria in test tube(**in vitro**) this is usually the path it follows

Bacterial growth curve

If a small number
of bacteria are inoculated
into a liquid nutrient
medium



Bacterial growth curve



Lag phase

No. of bacteria

1

Lag phase

When we put a bacterial in a medium it enters a state of adaptation, there will be **increase in size because**

- It is Metabolically active
- the medium is rich with nutrients

No. constant

& ↑ Size

Time/hrs

No. of bacteria

Log phase

Exponential phase

Start division

2

Log phase

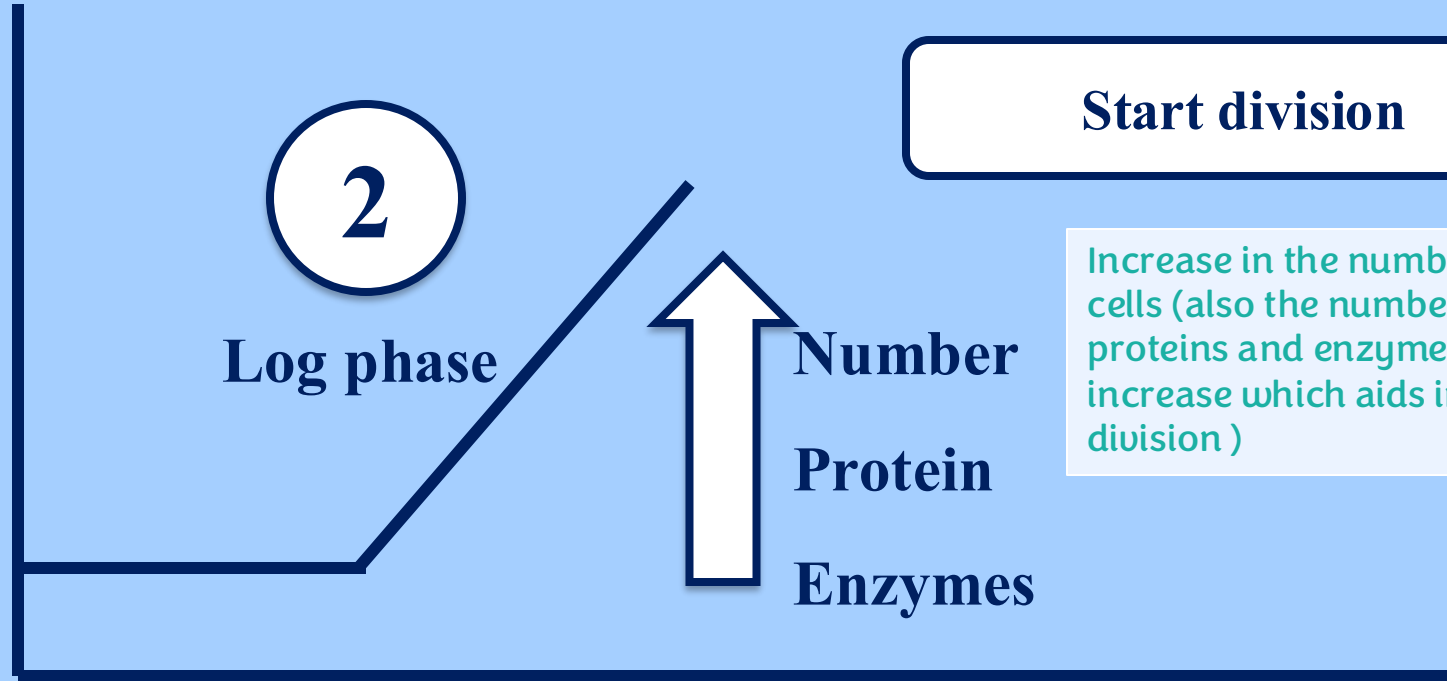
Number

Protein

Enzymes

Increase in the number of cells (also the number of proteins and enzymes increase which aids in division)

Time/hrs



Stationery phase

No. of bacteria

3

Stationary phase

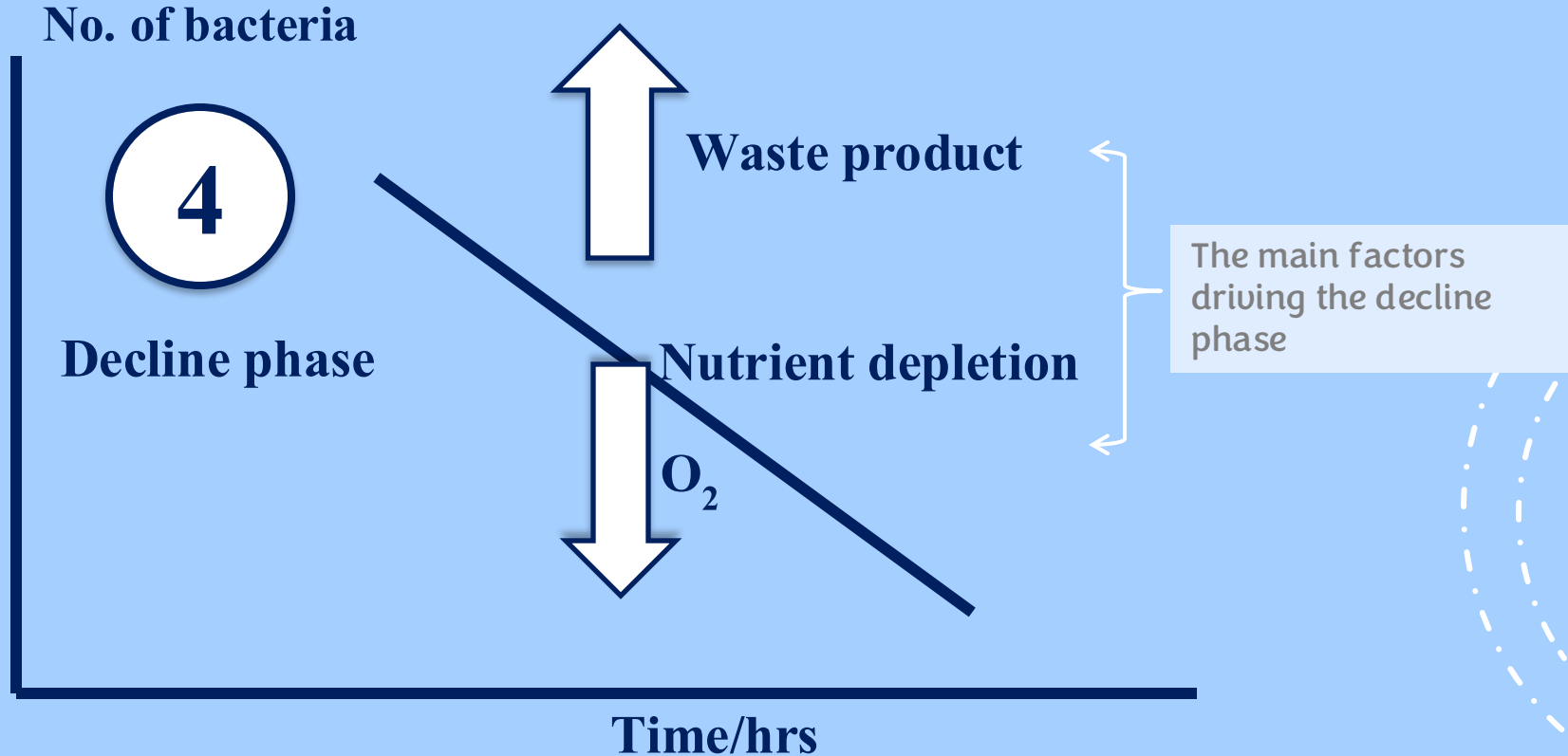
Constant number

No. of divisions = No. of deaths

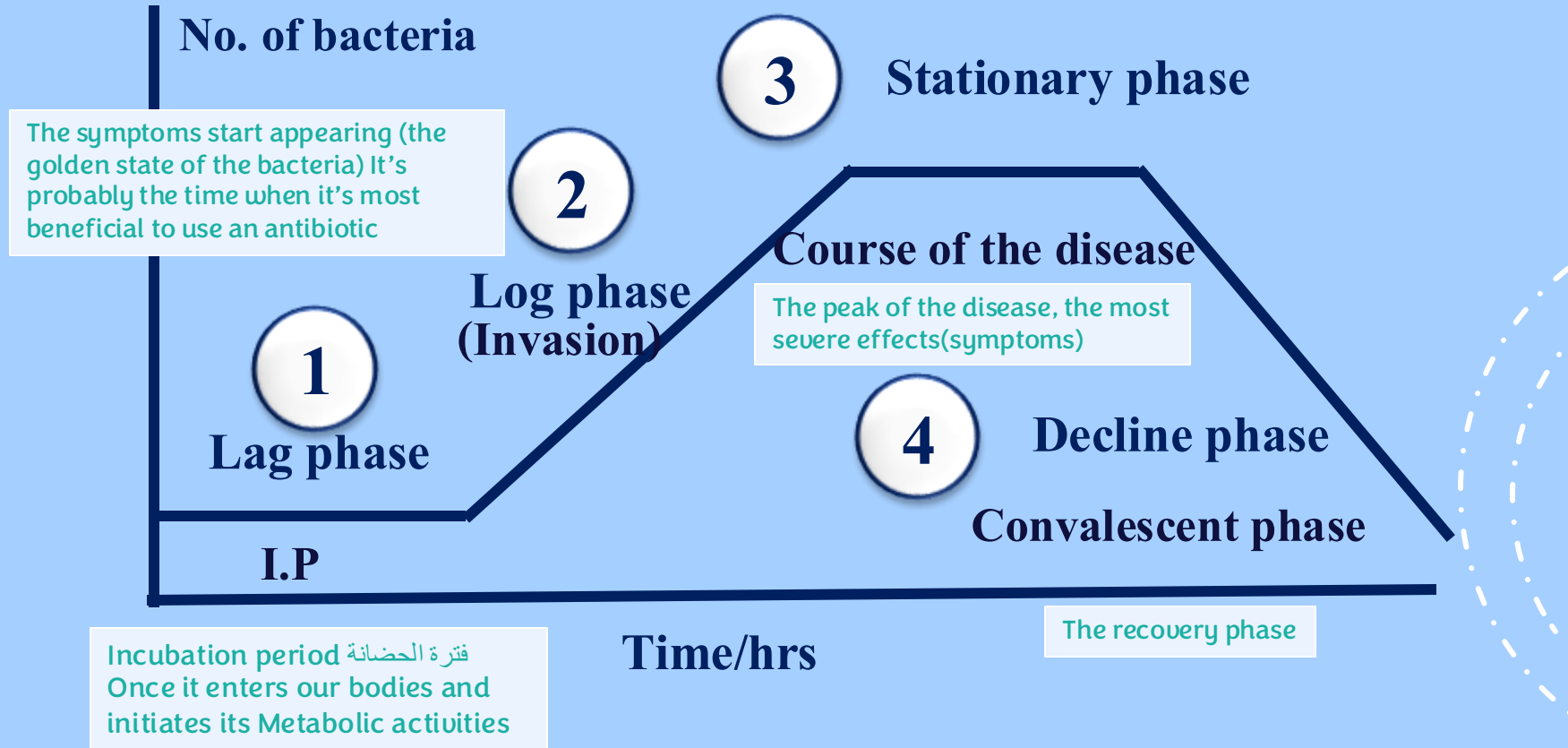
Nutrient depletion & Waste
accumulation and toxic materials

Time/hrs

Decline phase



Bacterial growth: In Vitro Vs. In Vivo



Bacterial growth requirements

A) Nutrition

B) Gaseous

C) Temperature & pH

A) Nutrition

Two types of bacteria when talking about **modes of nutrition**:

1- Autotrophic

auto = self

Trophic=nutrition

It produces its own food

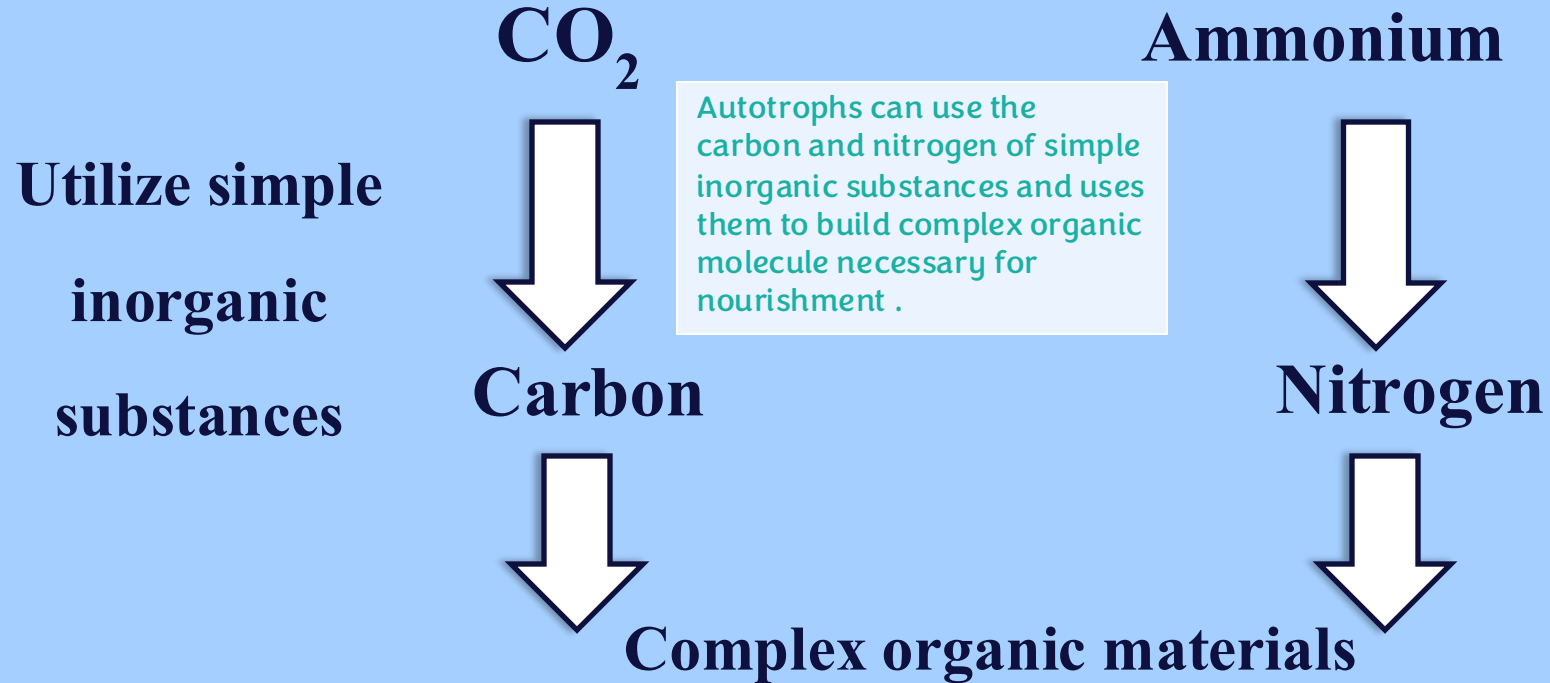
2- Heterotrophic

hetero = different

Trophic = nutrition

It relies on other beings for their food

Autotrophic



Heterotrophic: Need preformed organic substances

e.g. sugars, proteins etc.

Saprophytic

محات

Medically not important
(it doesn't influence our
bodies)



Lives on dead materials



Medically unimportant

Parasitic

Don't mix it with
parasites , those are
named parasitic
because they rely on
other beings for food
supply



Lives on living host



Medically important

Growth Requirements

- Gaseous requirements

According to O₂ requirement, bacteria are classified into 5 groups

Oxygen requirement

Types depending on oxygen requirement:

- 1) Obligate aerobes**
- 2) Obligate anaerobes**
- 3) Facultative anaerobes**
- 4) Micro-aerophiles**
- 5) Aero-tolerant anaerobes**

Bacterial Respiration

Glucose catabolism

Energy production

Aerobic respiration

(O₂)

Anaerobic respiration

(No O₂)



energy

1- Obligate aerobes (Aerobic respiration)

Presence of O₂



Growth

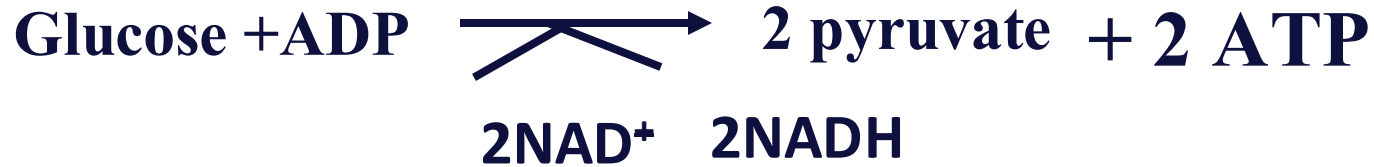
Absence of O₂

No growth

e.g. Pseudomonas aeruginosa

1- Obligate aerobes (Aerobic respiration)

Glycolysis



Oxidative phosphorylation



38 ATP

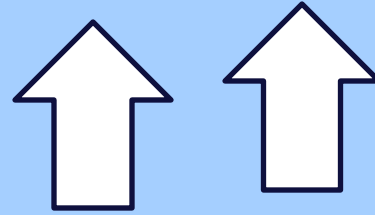
1- Obligate aerobes

Highly toxic molecules

Superoxide

Hydrogen peroxide

Usually obligated aerobes get rid of the metabolic toxic materials by those enzymes (there accumulation can cause cell injury)



Superoxide dismutase

Catalase

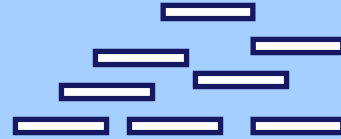
2- Obligate anaerobes

Oxygen is toxic for them

Presence of O₂

No growth

Absence of O₂



Bacteroides fragilis

2- Obligate anaerobes(Anaerobic respiration)



O_2



Other pathway

4 ATP

2- Obligate anaerobes(Anaerobic respiration)

The organism uses inorganic molecules

Nitrate

Sulfate

CO₂

Accept H⁺

13 ATP + 4 ATP

17 ATP

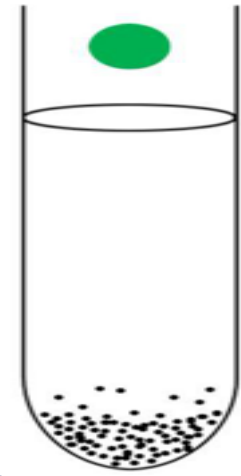
In obligated anaerobes H⁺ will be acquired by other receptors different of what we know in aerobic respiration (oxygen is the acceptor), and hence making a **fewer amount of ATP**

Lack

Superoxide dismutase

Catalase

Obligate
Anaerobe



3- Facultative anaerobes

Facultative anaerobes can withstand the presence or absence of oxygen but the growth will be rapid in the presence of oxygen

Presence of O₂

Absence of O₂



Growth

Growth

Rate of growth

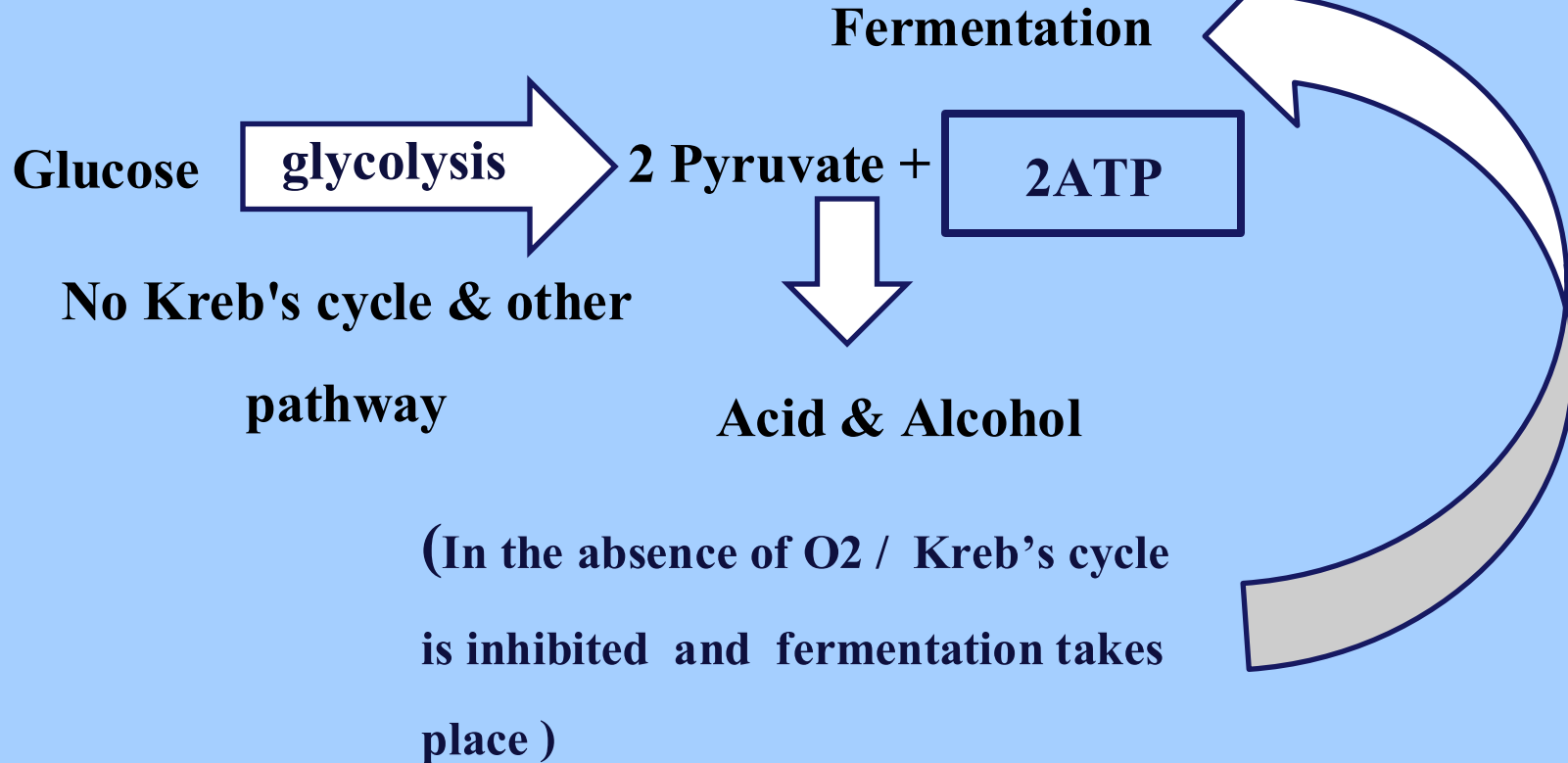


Full aerobic Respiration
(Glycolysis, kreb's cycle,
oxydative phosphorylation)

Most bacteria

Alcohol/acid fermentation

3- Facultative anaerobes/Anaerobic condition



4- Micro-aerophilic

Presence of O₂

21%

The normal proportion in air

No growth

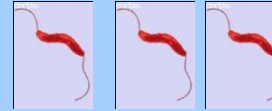
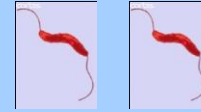


superoxide & H₂O₂

Low superoxide

dismutase & catalase

Low O₂



2-10% O₂

Growth

It can't survive with a high percentage of oxygen because they have a small amount of Superoxide dismutase and catalase to handle the toxic byproducts

Campylobacter

Helicobacter

5- Aero-tolerant anaerobes

Presence of O₂



Growth

They do not use O₂ for growth, but they tolerate it because they have a low Superoxide dismutase

Absence of O₂



Growth

They grow equally well via fermentation

Lactobacillus

Growth Requirement: CO₂ requirements

CO₂ (0.03%)

Present in the air is
sufficient for most bacteria

CO₂ (5-10%)

(Capnophiles)

Neisseria

Brucella

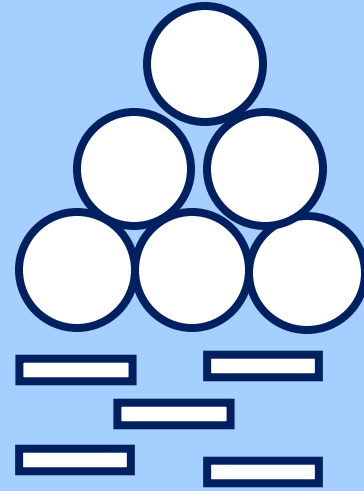
Needs higher percentage of CO₂ than
other organisms

Growth Requirement: Hydrogen ion (pH)

pH (7.2 – 7.4)
(Most bacteria)

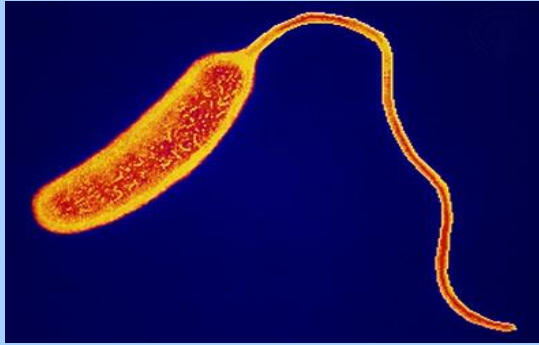
Neutrophiles

Most of bacteria



Hydrogen ion (pH)

Alkaline (pH 9)



Vibrio Cholerae

Alkaliphiles

Acidic (pH 4)



Lactobacilli

Acidophiles

Growth Requirement: Temperature

Mesophiles

(20 – 45)

(Most bacteria)

psychrophiles

(0 – 15)

Thermophiles

(55– 65)

Quiz time



For any feedback, scan the code or click on it.



Versions	Slide # and Place of Error	Before Correction	After Correction
V0 □ V1			
V1 □ V2			

Additional resources:

اللهم وفقني لما تحب وترضى، وخذ بيدي
للخير حيث كان، وبارك لي في خطواتي
وأيامي.

رسالة من الفريق العلمي: