Microbiology

Modified 5

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Date : Oct 2025

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Lecture 3

Bacterial Growth& physiology

Bacterial Growth

- **Definitions**
- **B. Reproduction**
- **culture media**

A. Definition

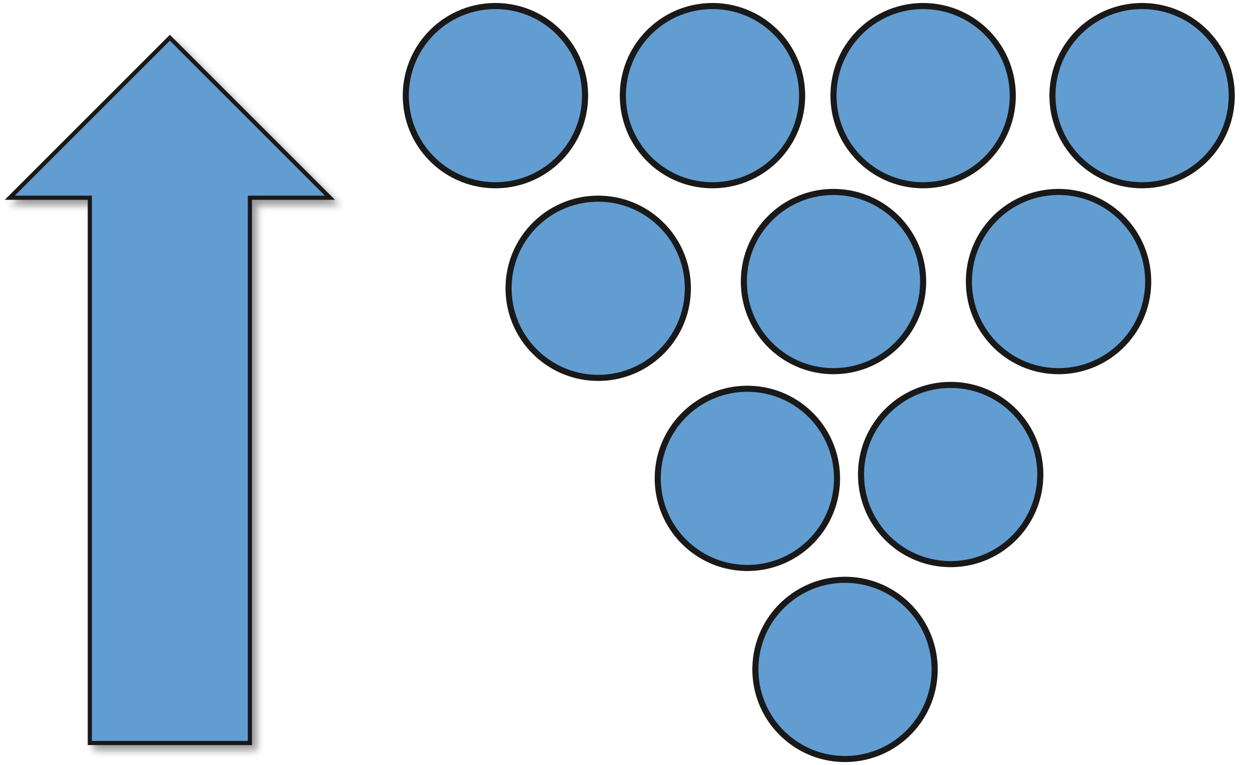
B. Classification

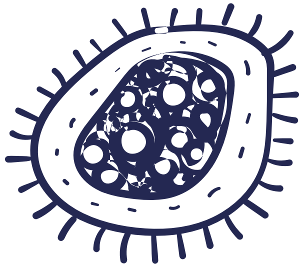
C. Types

- **Bacterial growth curve**

Bacterial Growth: Definition

Bacterial growth: is defined as the increase in both **Size** &**Number** of organism



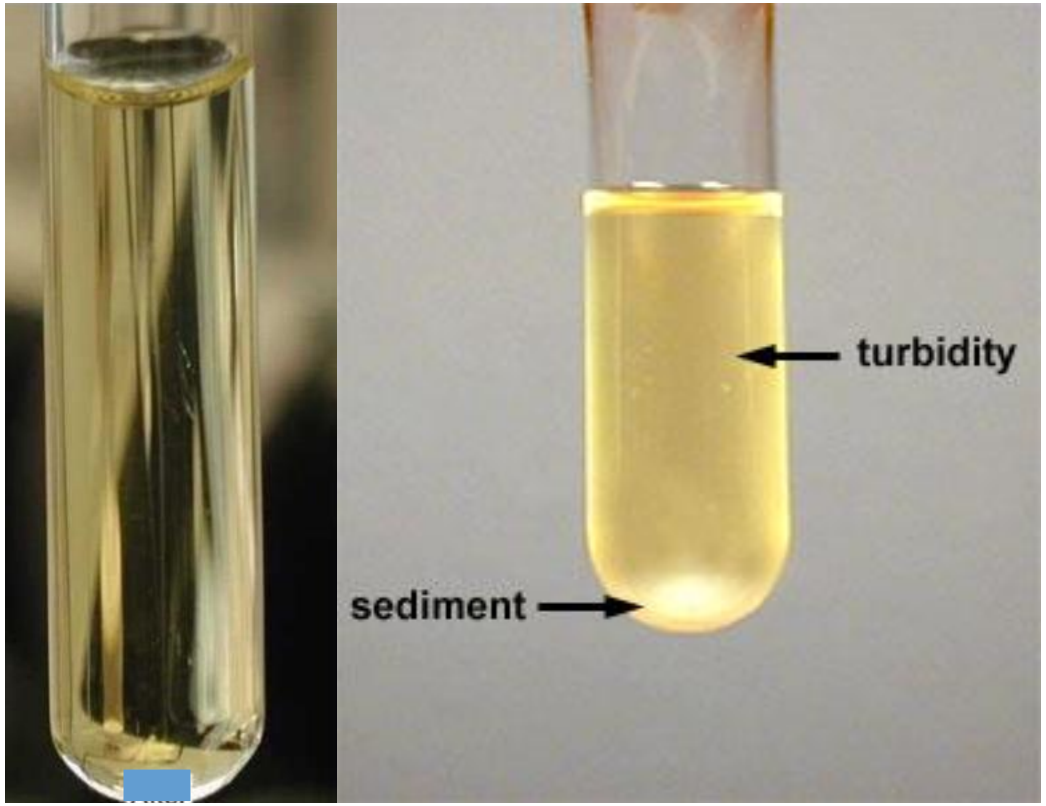


1) Bacterial Growth

Bacterial growth can be detected in two primary ways:

1. Turbidity of the fluid media

- If bacteria are cultured in a clear liquid broth and left for several hours, the broth will become **cloudy or turbid**, which indicates that **bacterial growth** has occurred.



1) Bacterial Growth

2) Colonies on solid media

(Macroscopic product)

- Colonies are referred to as macroscopic products because they are **visible** aggregates of bacteria that can be seen with the **naked eye**.

2) Colony (Macroscopic product)

Single bacterium

Cultured on solid media

After 20-30 division

By Binary fission

Colony

So the appearance of colony will be after 20-30 division of single bacterium

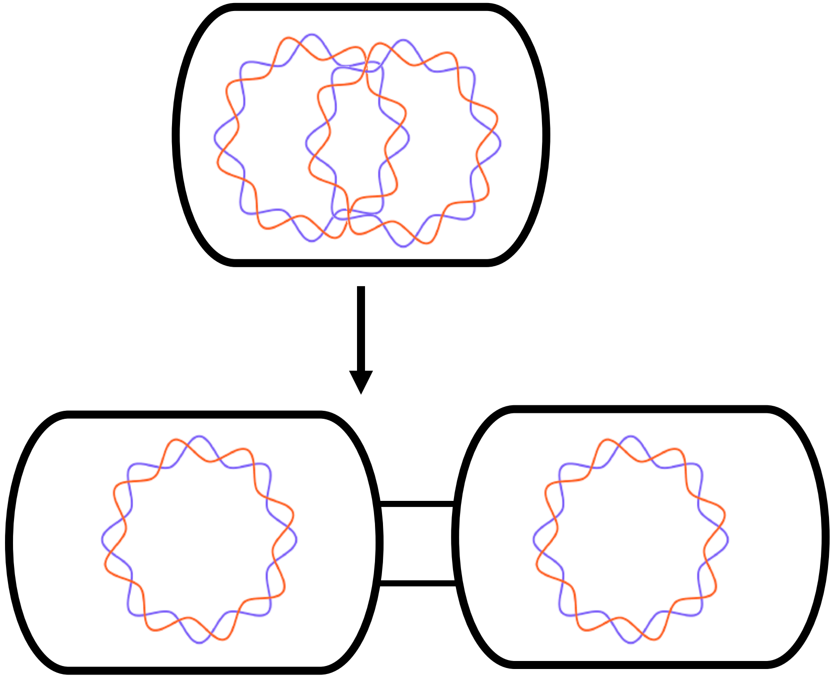
2) Colony

After 20–30 division

Binary fission

1 Million

(2^{20})



This exponential growth (2^n) occurs because each division yields two daughter cells:

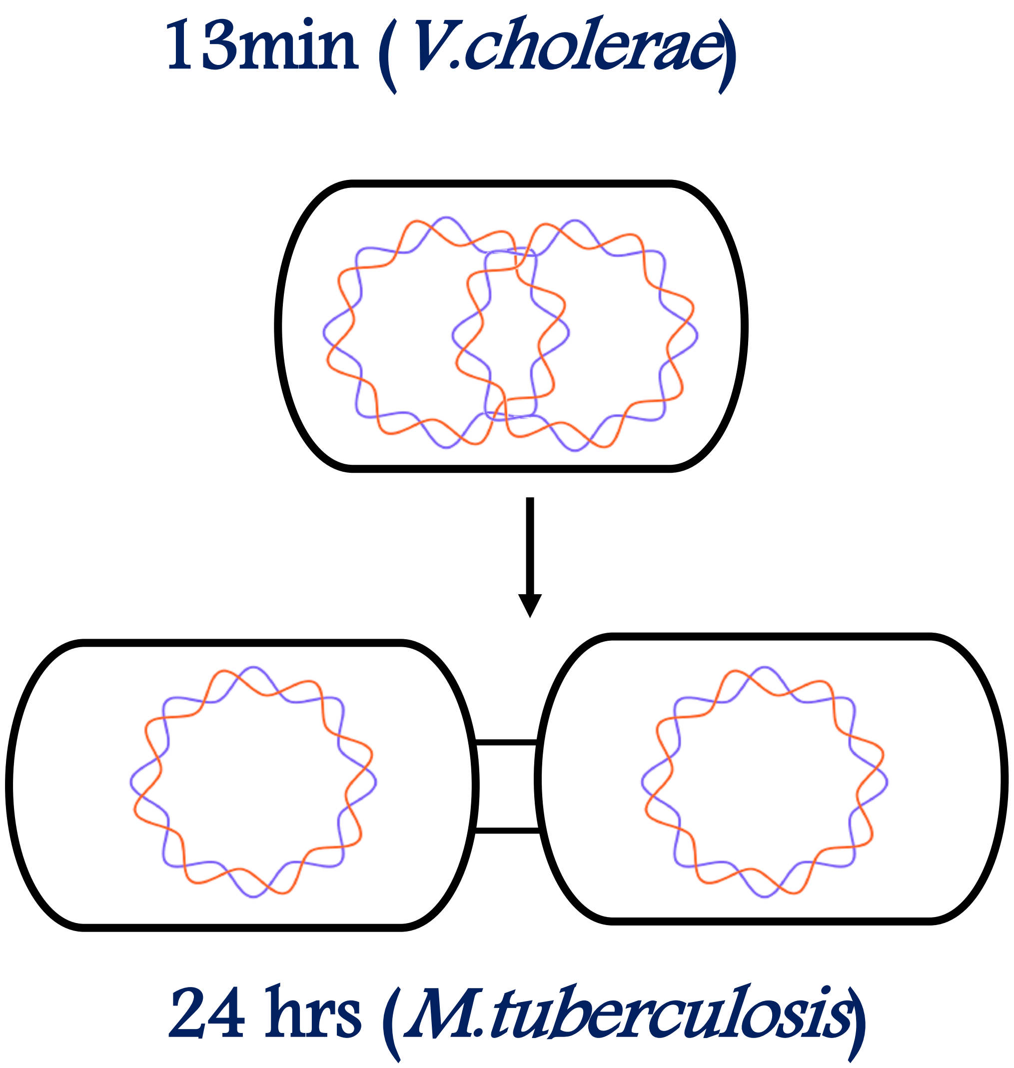
- 1st division: 2 cells
- 2nd division: 4 cells
- 3rd division: 8 cells
- 4th division: 16 cells5th division: 32 cells, and so on.

3) Generation time (doubling time)

- **Generation time (or doubling time):** is the time it takes for a bacterium to complete one division.

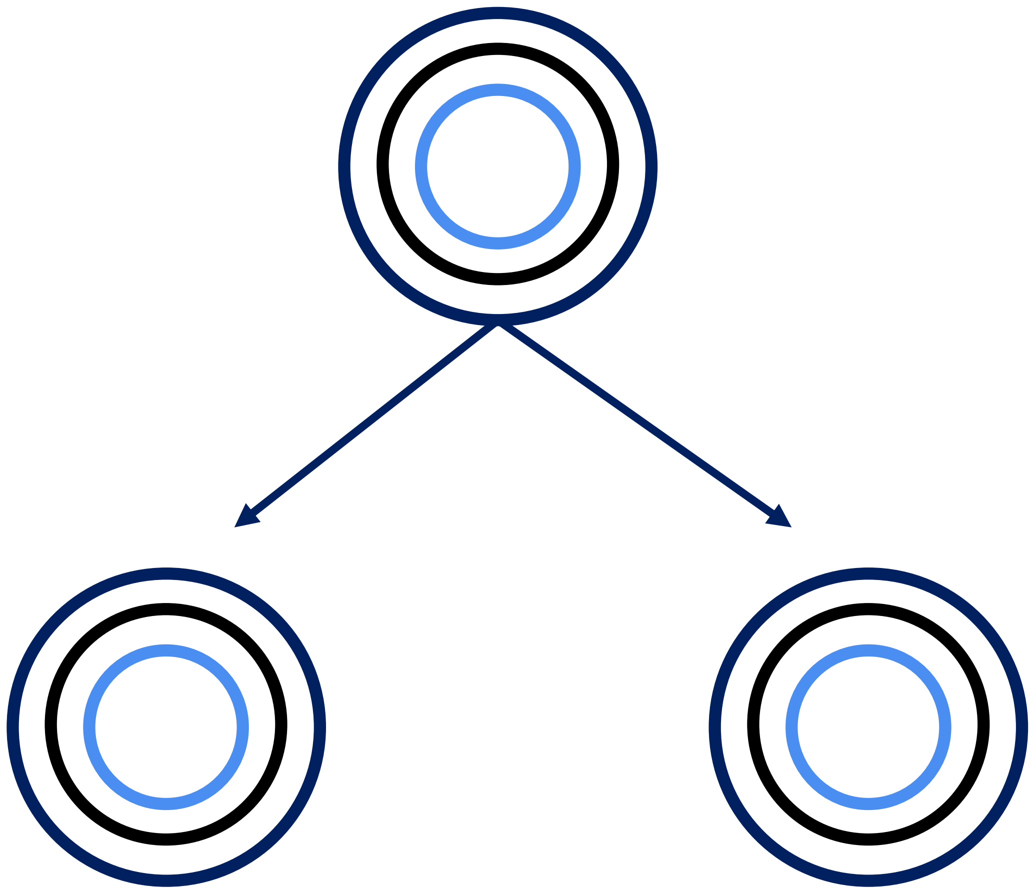
- **This time varies significantly between different microorganisms.**
  - Some bacteria are **very fast**, such as *Vibrio cholerae*, which can divide every 13 minutes

  - Other bacteria are **very slow**, such as *Mycobacterium tuberculosis*, which requires approximately 24 hours to complete a single division.



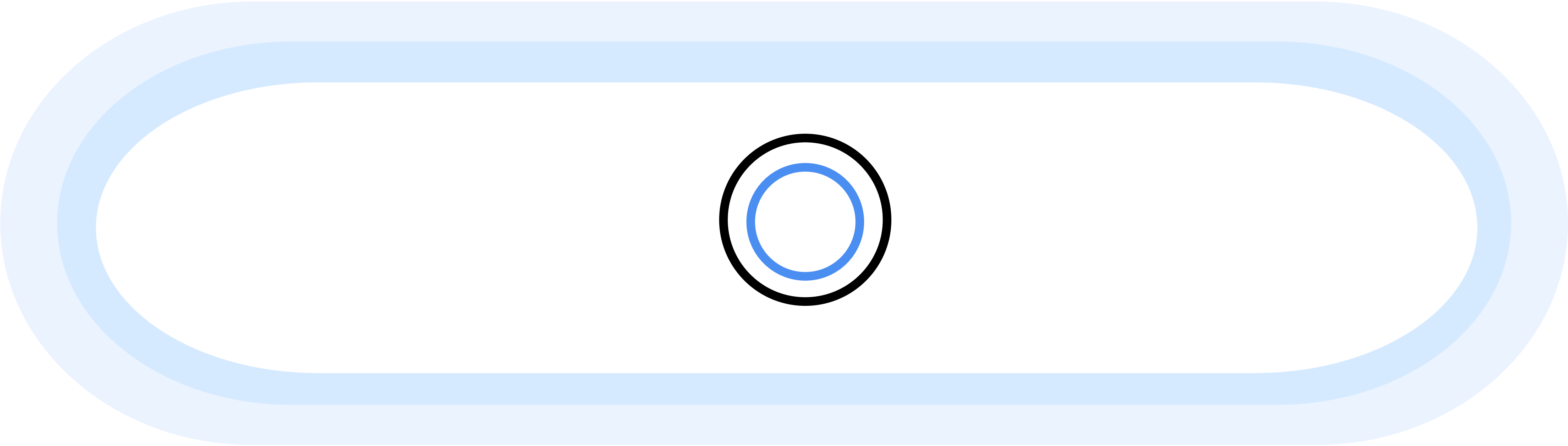
Bacterial Reproduction

Binary fission



Bacterial Reproduction

1 Elongation



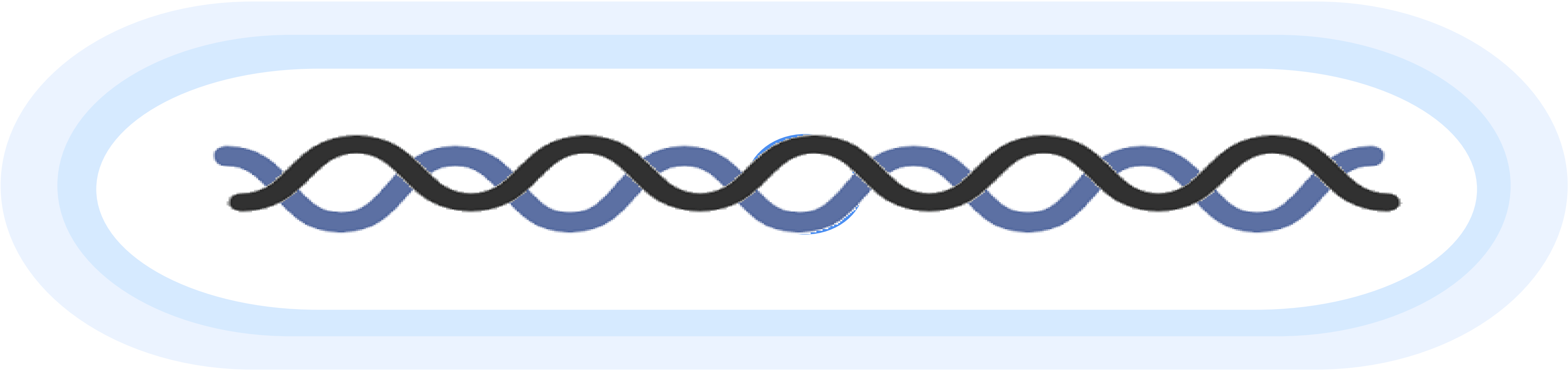
Bacterial Reproduction

- The bacterial cell contains a single, circular, double-stranded DNA molecule.

  For simplicity we will assume that is double helical stranded

1

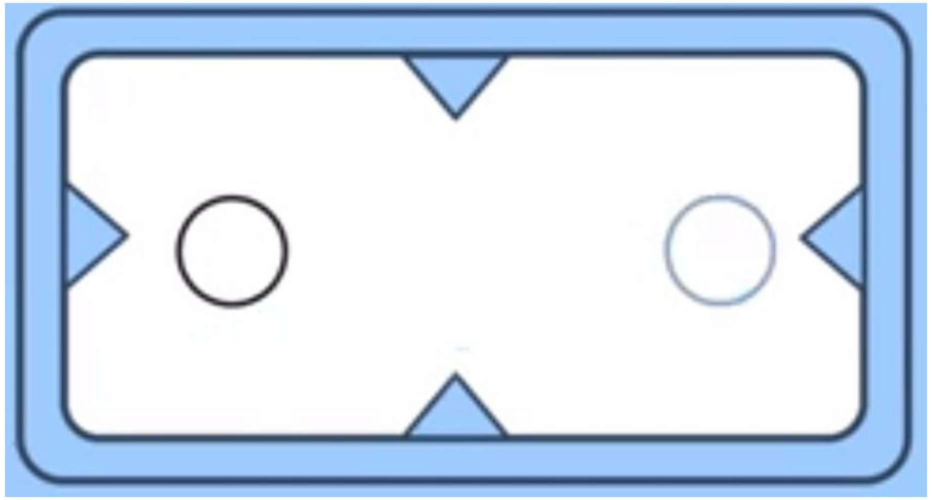
Elongation : The bacterium cell, which contains a single, circular, double-stranded DNA, begins to elongate. This elongation typically occurs in one direction to ensure the resulting daughter cells are the **same size as the parent**.



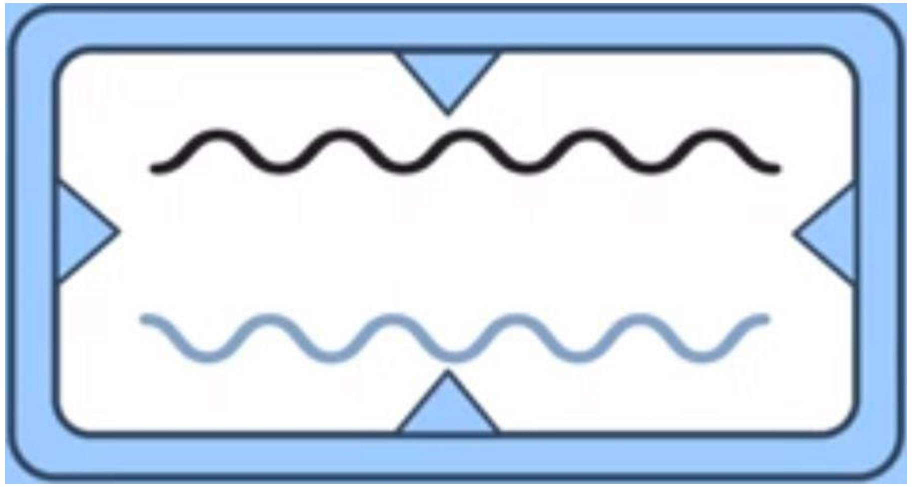
Bacterial Reproduction

2

Separation of 2 strands: The two strands of the circular DNA separate. Each single strand attaches to a **mesosome**. The mesosome contains enzymes, equivalent to those in mitochondria, that **unwind the double-stranded DNA.**



Original shape of DNA

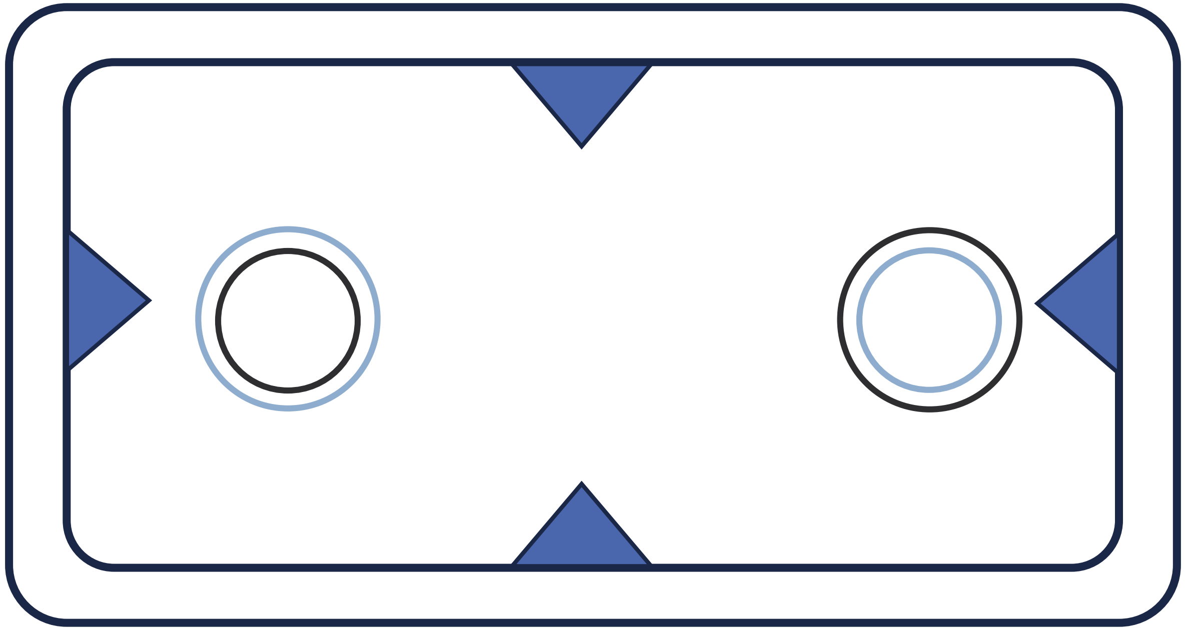


Assumed shape of DNA

Bacterial Reproduction

3 Separate ssDNA & become dsDNA

- The separated single-stranded DNA (ssDNA) molecules, which are now on opposite sides of the cell, each act as a template to build a new complementary strand, resulting in two identical double-stranded (dsDNA) circular molecules.



Bacterial Reproduction

4 Formation of division septum

- A division septum (specifically the septal mesosome) begins to form in the middle of the elongated cell.

Bacterial Reproduction

5 Cell separation

- The septum constricts (narrows) progressively until it completely separates the cell, resulting in two identical daughter cells.

Bacterial culture media

Bacteria grow
(In vitro)

Need nutrients for growth

Artificial

- **Culture media** is an artificial nutrient medium used to grow bacteria in **vitro** (outside a living organism).
- It provides the **necessary nutrients** for bacterial growth, such as proteins, salts, and sugars.

Purpose

1

Study Properties

The primary purposes for culturing bacteria are:

- **Detection**: To determine if an infection is bacterial or non-bacterial.
- **Study Properties**: To observe and study the characteristics of the bacteria.

Purpose

2

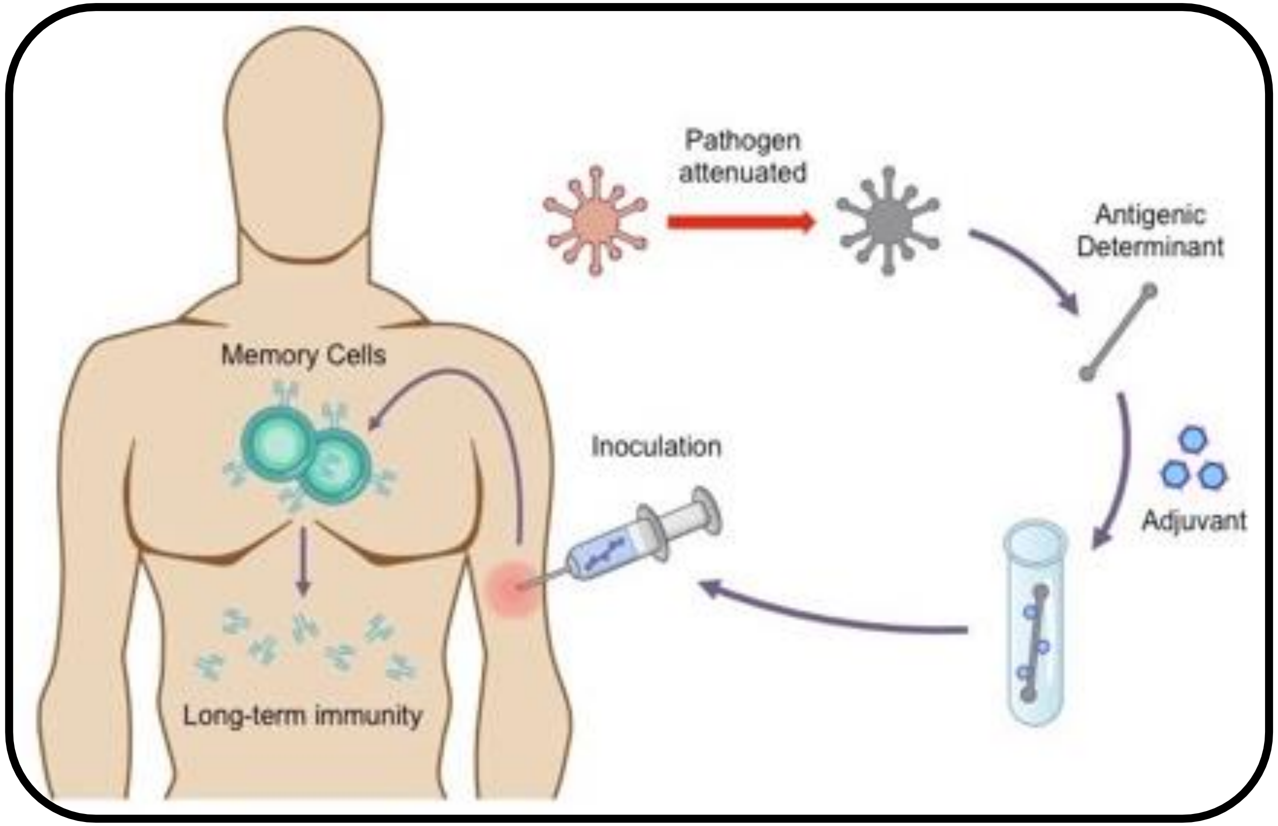
Isolation & diagnosis (Causative agent)

- To isolate the specific bacterium and identify it as the causative agent of a disease.

Purpose

3

Prepare vaccine & Other product

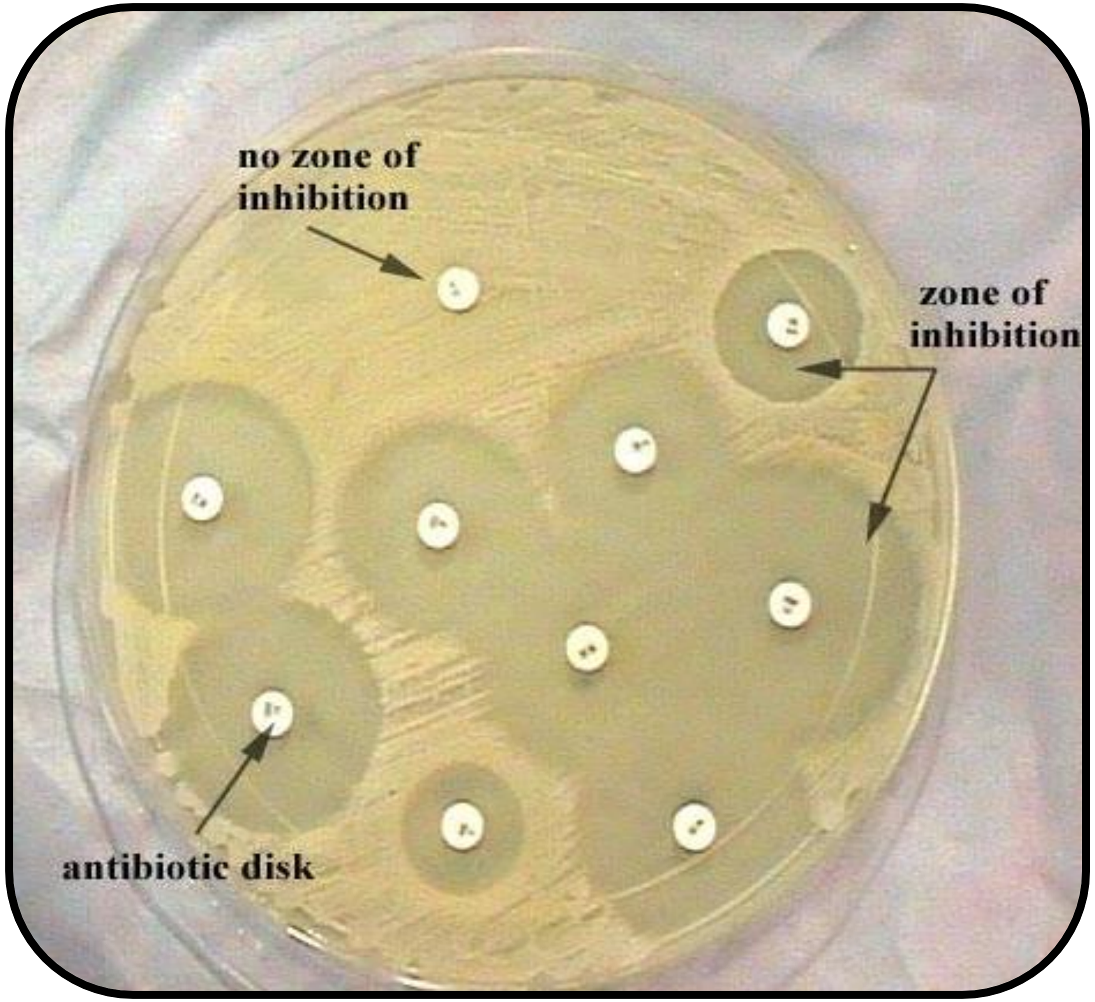


- To grow bacteria for the creation of vaccines or other products (e.g., extracting Streptokinase from Streptococcus pyogenes to dissolve blood clots).

Purpose

4

For Selection proper antibiotics



Classification of media

Liquid

Used in test tubes and referred to as **broth**.

Solid

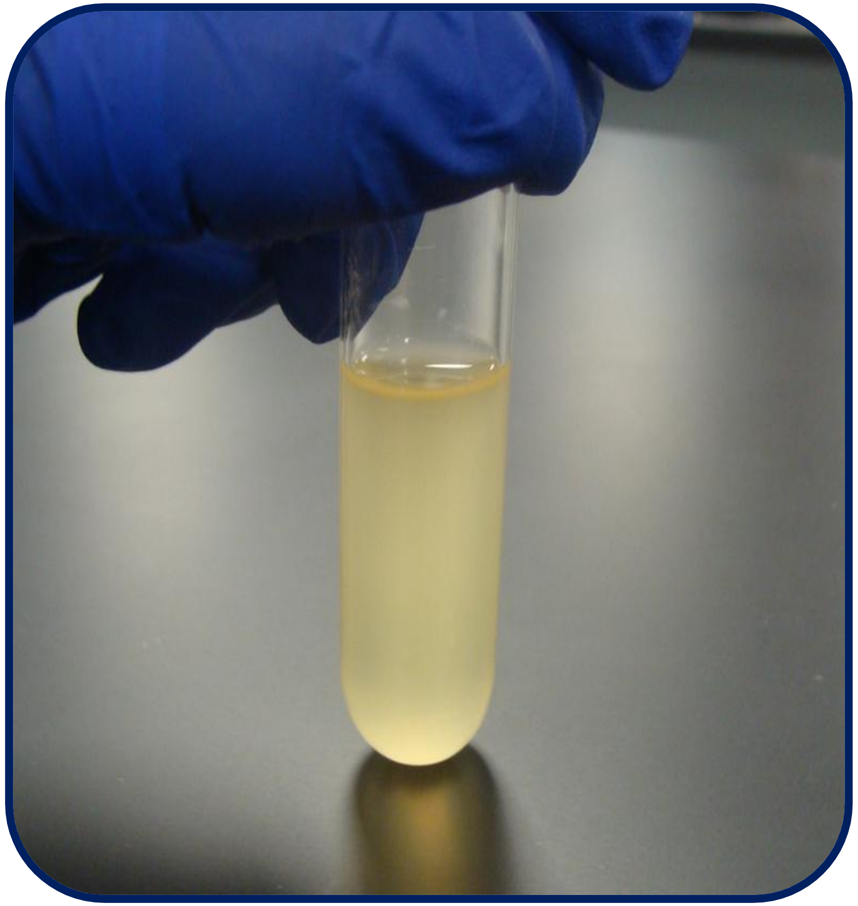
Have a Jell-like structure and are poured into **Petri dishes**.

Types of media

1) Simple media
2) Enriched media
3) Selective media
4) Differential media

Simple media

Basic requirement for growth of most bacteria



Simple media

A) Peptone water

Peptone + 0.5% NaCl

- Enhancement : supports bacterial growth
- serves as a base for Sugar media

Simple media

B) Nutrient broth

Meat extract

Enhancement

Simple media

C) Nutrient agar plate

Nutrient broth + 2% agar

agar

(Seaweed)

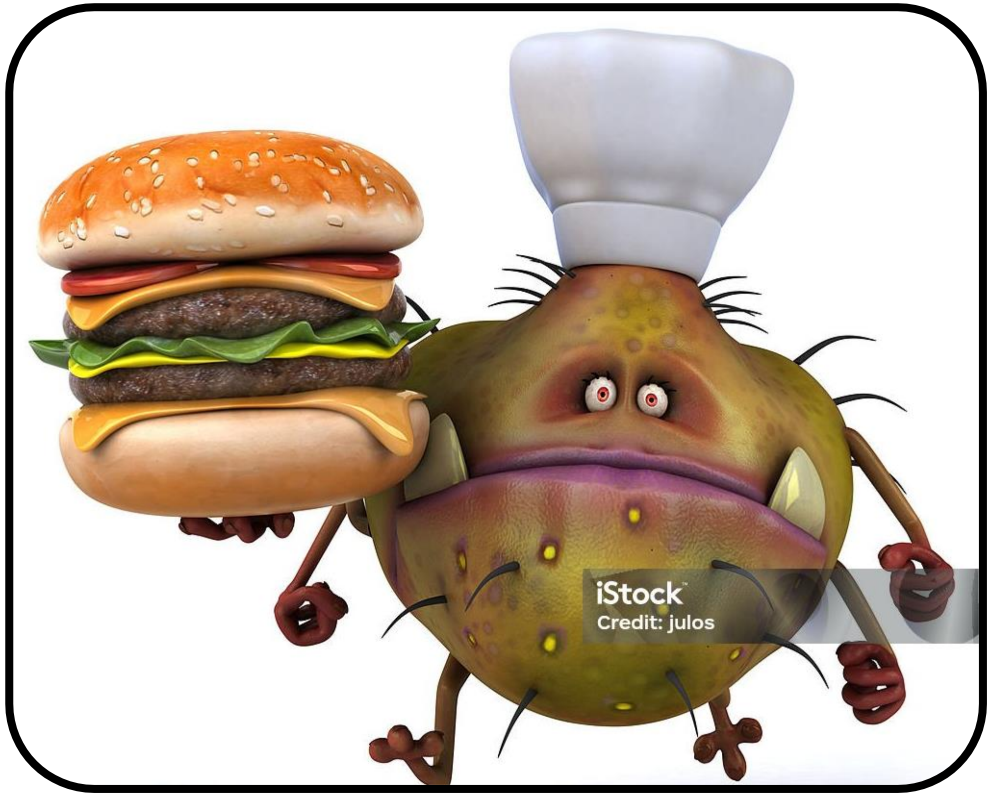
- This is a solid medium created by adding **2% agar-agar** (a substance derived from seaweed) to **nutrient broth**.
- The mixture is boiled (approx. 100 C), poured into Petri dishes, and solidifies at room temperature to a semi-solid, Jell-like consistency.
- It is suitable for growing bacteria like **Staphylococcus aureus.**

Staph. aureus

Enriched media

Fastidious bacteria

Need blood, serum for growth



- **Fastidious bacteria:** organisms that are "demanding" or "spoiled" and require additional nutrients beyond what simple media offers, such as **blood** or **serum**.

Enriched media

A) Blood agar

Nutrient agar heated at 45°C
(semisolid)
+ sheep blood

- Sheep blood is preferred to avoid the presence of antibiotics or antibodies that might be in other blood sources and could affect results.

Enriched media

A) Blood agar

Streptococci

e.g. Strept. Pyogenes

& Strept. pneumoniae

Hemolysis on blood agar:

A. Complete (beta) hemolysis:

- *Staphylococcus aureus*
- *Streptococcus pyogenes*

*The bacteria completely destroy the RBCs, creating a **clear zone around the colony**.*

C. No (gamma) hemolysis:

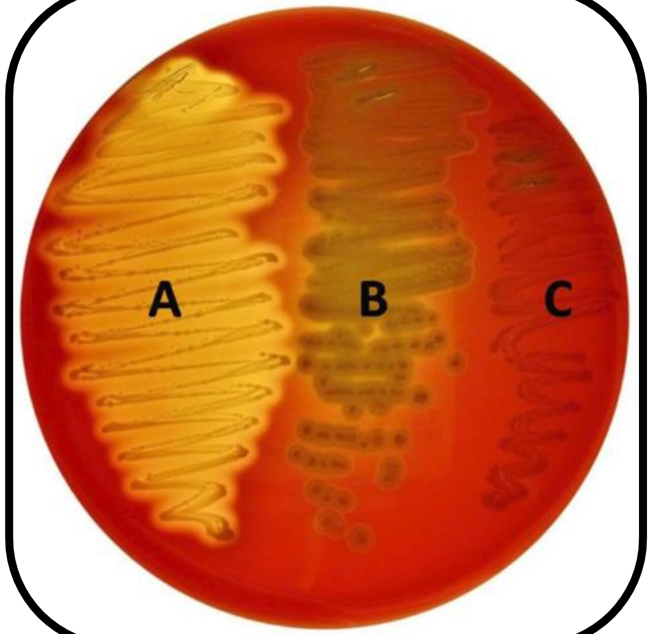
- *Enterococci.*

*The bacteria do not cause any hemolysis, and the media remains **unchanged**.*

B. Partial (alpha) hemolysis:

- *Streptococcus viridans*
- *pneumococci.*

*The bacteria partially break down the RBCs, resulting in a **greenish discoloration** around the colony.*



Enriched media

B) Chocolate agar

Nutrient agar heated at 100°C, add blood

Hemoglobin (Hb) → Heat → Hematin (Chocolate)

- The high heat breaks down the RBCs, releasing hemoglobin (Hb), which is further broken down into **hematin**.
- **Hematin** gives the medium dark chocolate color, which is how it gets its name.

Enriched media

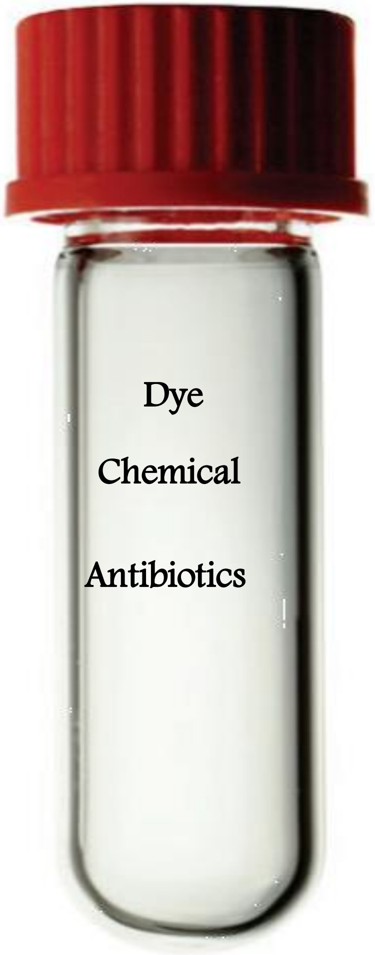
B) Chocolate agar

It is required for growing highly fastidious bacteria, such as **Haemophilus and Neisseria.**

Selective media

Allow a certain organism to grow (Selective) & inhibits the growth of others

- It is designed to **support** the growth of specific (**desired**) organisms while **inhibiting** the growth of other (**undesired**) organisms.
- This selection is achieved by adding substances **like dyes**, **chemicals**, or **antibiotics** that the desired organism is resistant to, **which helps in the identification process.**



Selective media

1. Lowenstein Jensen medium

Malachite green

Mycobacterium tuberculosis

- **Lowenstein Jensen Medium**: The **selective** ingredient is **malachite green**. This medium supports the growth of Mycobacterium species, especially ***Mycobacterium tuberculosis***, while inhibiting others.

Selective media

2. Blood tellurite agar

Potassium tellurite

C.diphtheriae

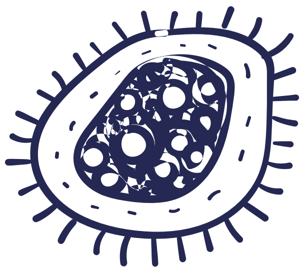
- **Blood Tellurite Agar:** The selective ingredient is **potassium tellurite**. This medium is selective for **Corynebacterium diphtheriae**.

Differential media

Selective + Indicator

Allow a certain organism to grow

Indicator to differentiate (change in visibly)



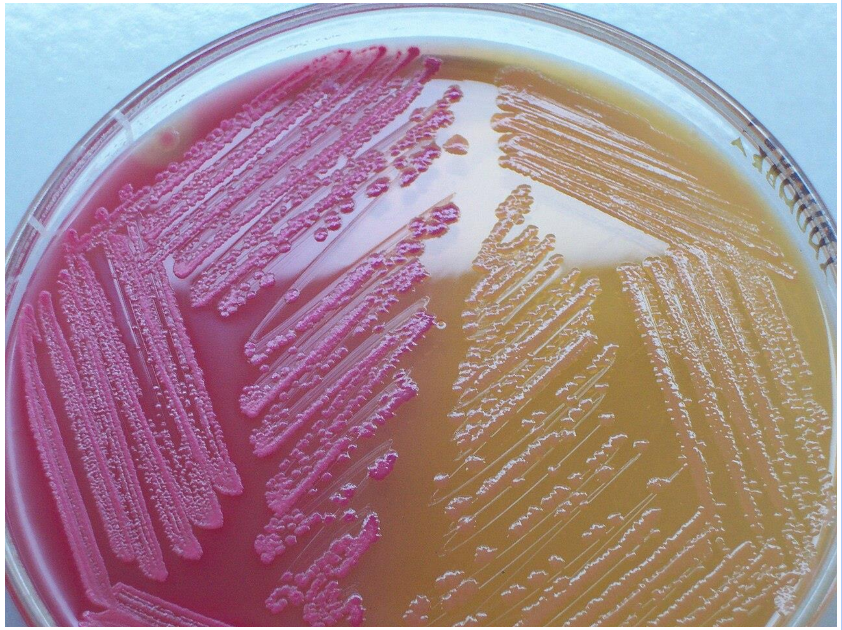
Differential media

1. MacConkey's agar

- **Selective Agent:** Peptone, Bile salts which are selective for Enterobacteriaceae.

- **Differential Components:**
  - **Lactose (the test sugar).**
  - **Neutral Red (the pH indicator).**

- **Mechanism:** It differentiates between bacteria that can ferment lactose and those that cannot:
  - **Lactose-fermenting bacteria:** Produce acid from lactose fermentation. This acid causes the neutral red pH indicator to turn **pink**.
  - **Non-lactose-fermenting bacteria:** Do not ferment lactose, so no acid is produced. The colonies remain **pale** (colorless).



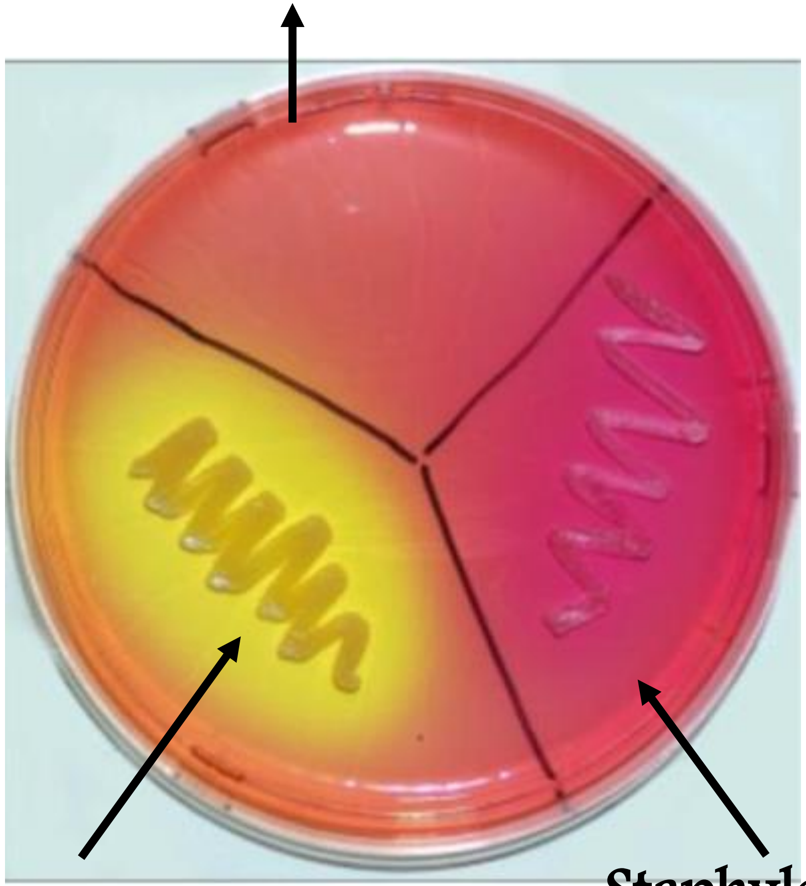
Pink Pale

Differential media

2. Mannitol salt agar

- **Selective Agent:** **High salt (7.5% NaCl)**, which is selective for *Staphylococcus* species.
- **Differential Components:**
  - ➢ **Mannitol** (the test sugar).
  - ➢ **Phenol Red** (the pH indicator).

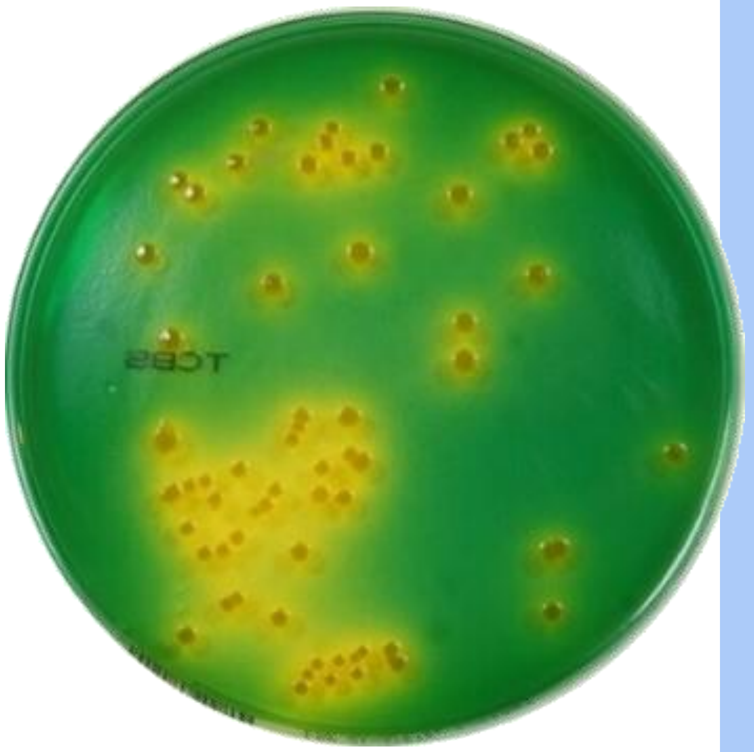
- **Mechanism:** It differentiates *Staphylococcus* species based on their ability to ferment mannitol.
  - ➢ ***Staphylococcus aureus***: **Ferments** mannitol, producing acid, which turns the phenol red indicator **yellow**.
  - ➢ ***Staphylococcus epidermidis***: **Does not ferment** mannitol, so the medium **remains pink**.



Differential media

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

- **Selective Agents**: **Thiosulfate, Citrate, and Bile**.
- **Differential Components:**
    - Sucrose (the test sugar).
    - Bromothymol Blue (the pH indicator).
- **Mechanism**: Used to differentiate Vibrio species.
    - **Vibrio cholerae: Ferments** sucrose, producing acid, which turns the bromothymol blue **indicator** yellow.
    - **Other Vibrio (e.g., parahaemolyticus): Do not ferment sucrose**, so the colonies remain green (the original color of the indicator).

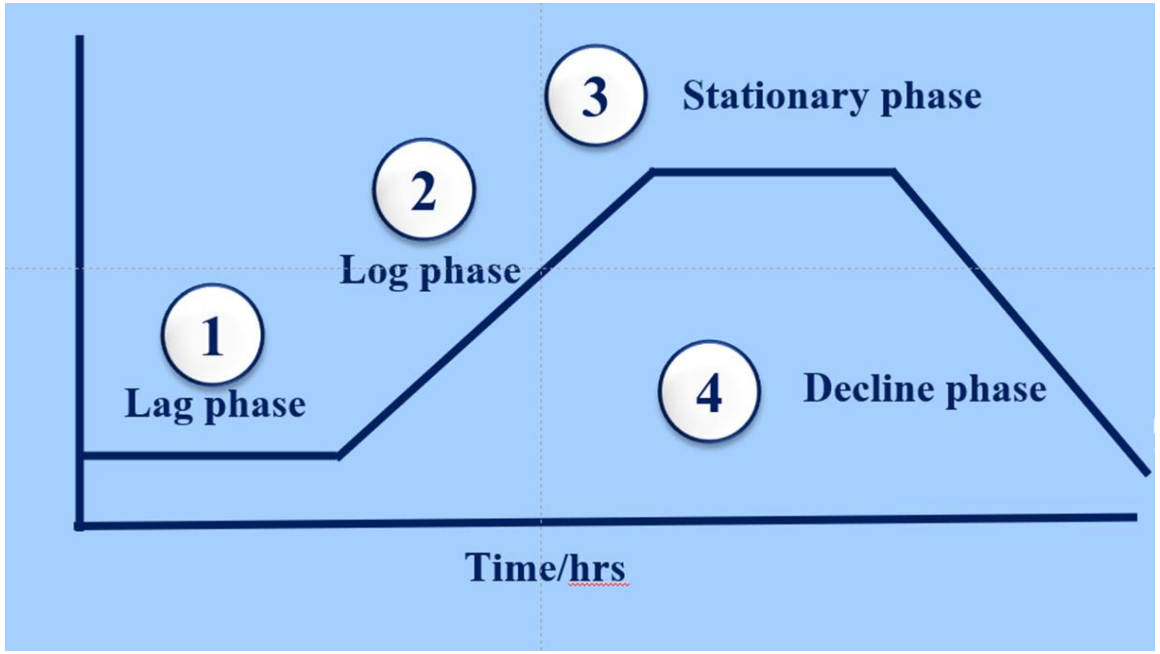


Bacterial growth curve

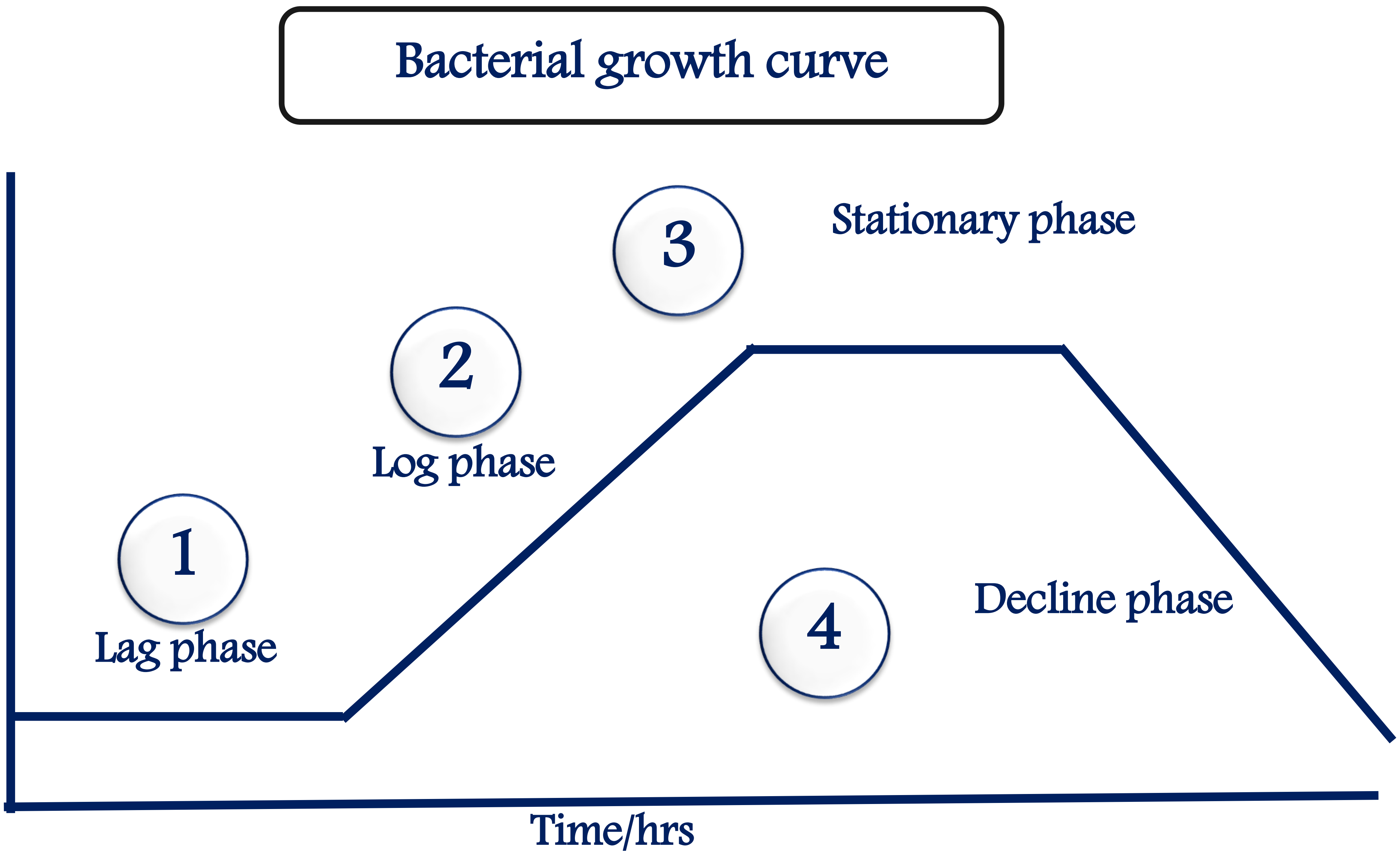
1) Lag phase
2) Log phase
3) Stationary phase
4) Decline phase

Bacterial growth curve

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



72h


Bacterial growth curve
3
Stationary phase
2
Log phase
1
Lag phase
4
Decline phase
Time/hrs

Lag phase

- The Lag phase is the **initial adaptation stage**.
- The **number** of bacteria **remains constant** (no division is occurring).
- The bacterial cells **increase** in **size** as they adapt to the new medium, synthesize necessary proteins and enzymes, and feed on available nutrients.

No. of bacteria

1

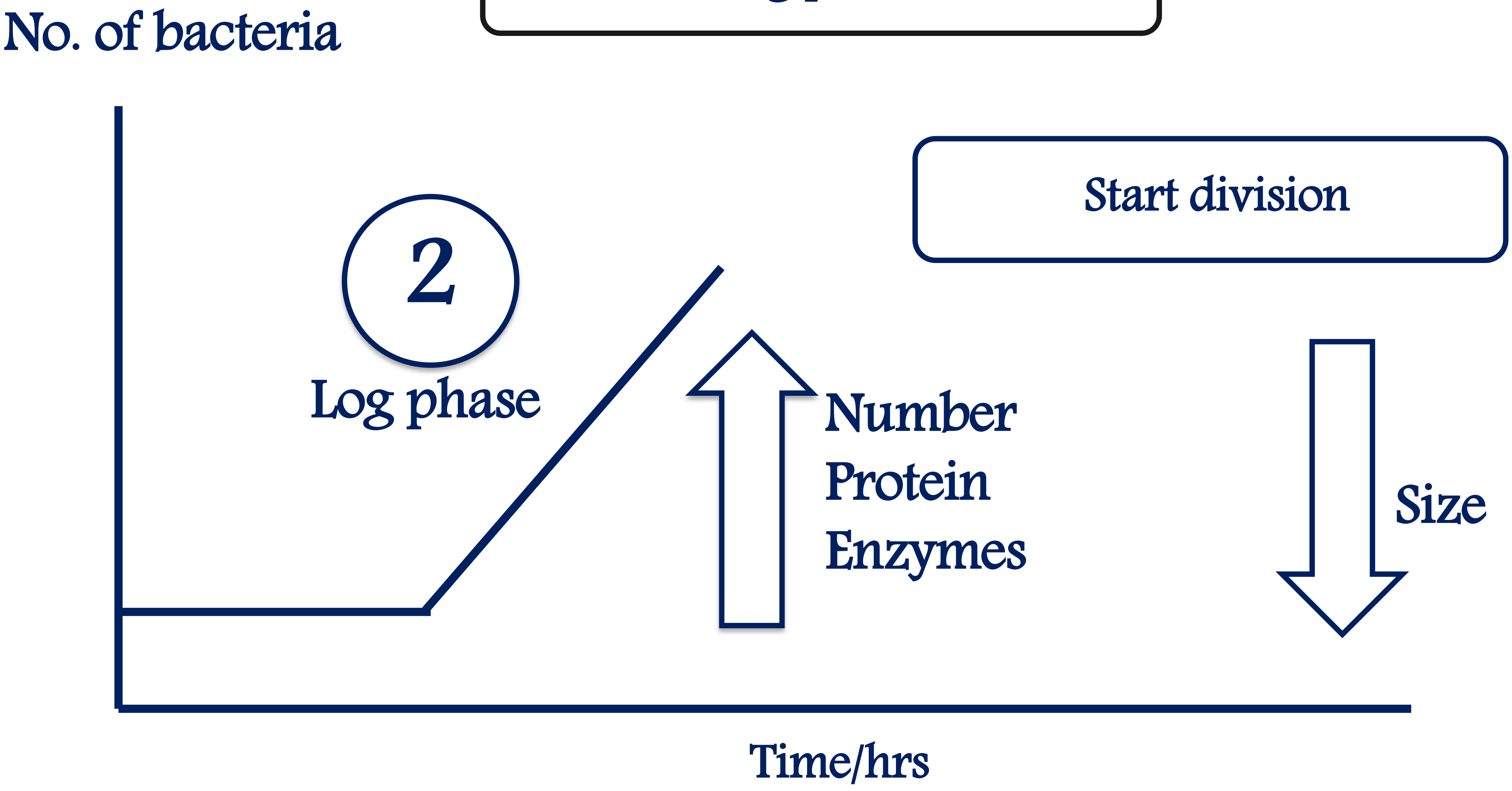
Lag phase

No. constant

& ↑ Size

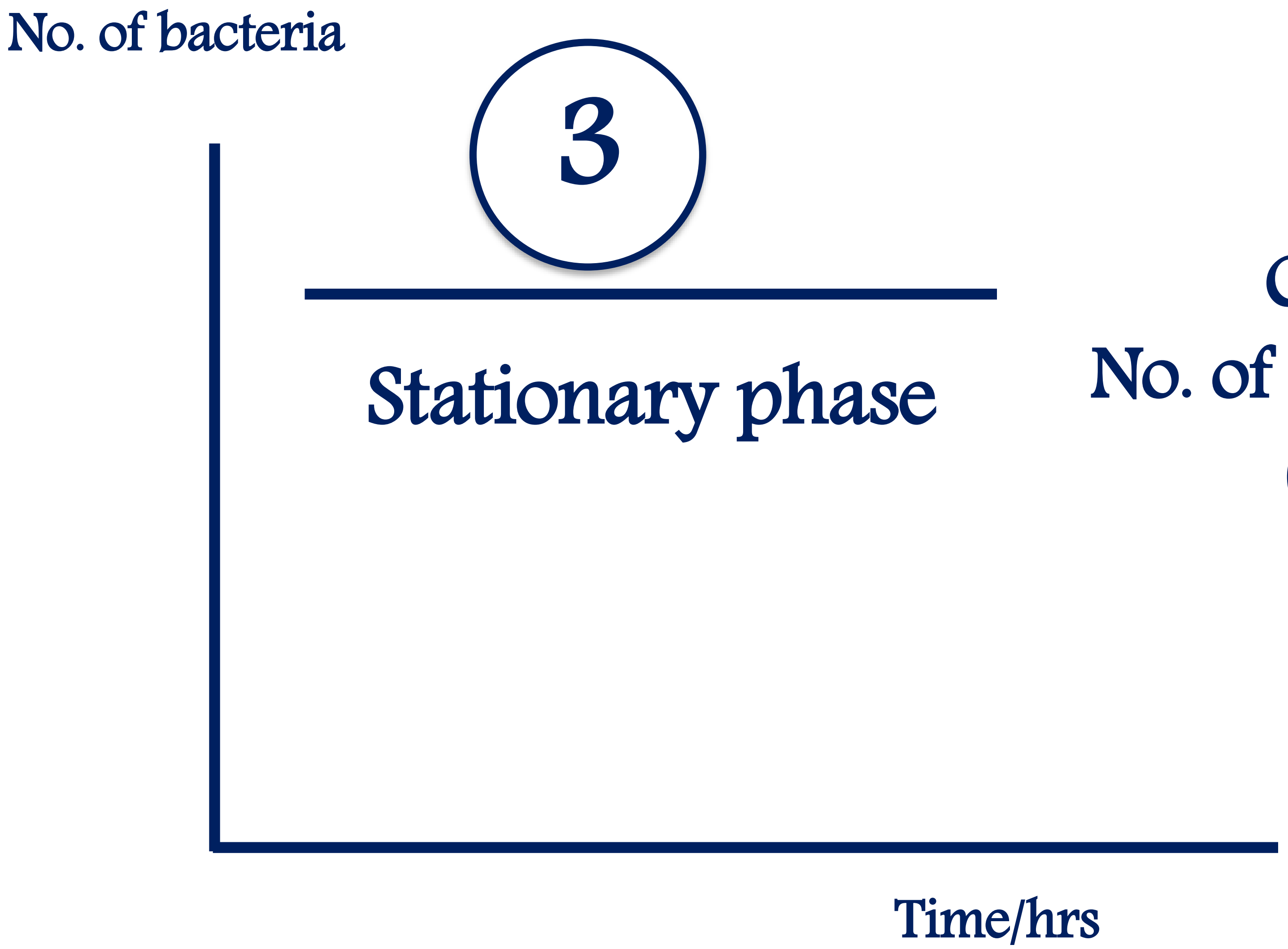
Time/hrs

Log phase


No. of bacteria
2
Log phase
Start division
Number
Protein
Enzymes
Size
Time/hrs

- In the Log phase (exponential phase), bacteria start dividing.
- There is an exponential increase in the number of bacteria, protein, and enzymes.
- The **size** of individual cells **decreases** as the large cells from the lag phase begin to **divide**.
- This is considered the "**golden phase**" for the bacteria and is the primary stage where **antibiotics are effective** (as they target growth and division).

Stationary phase

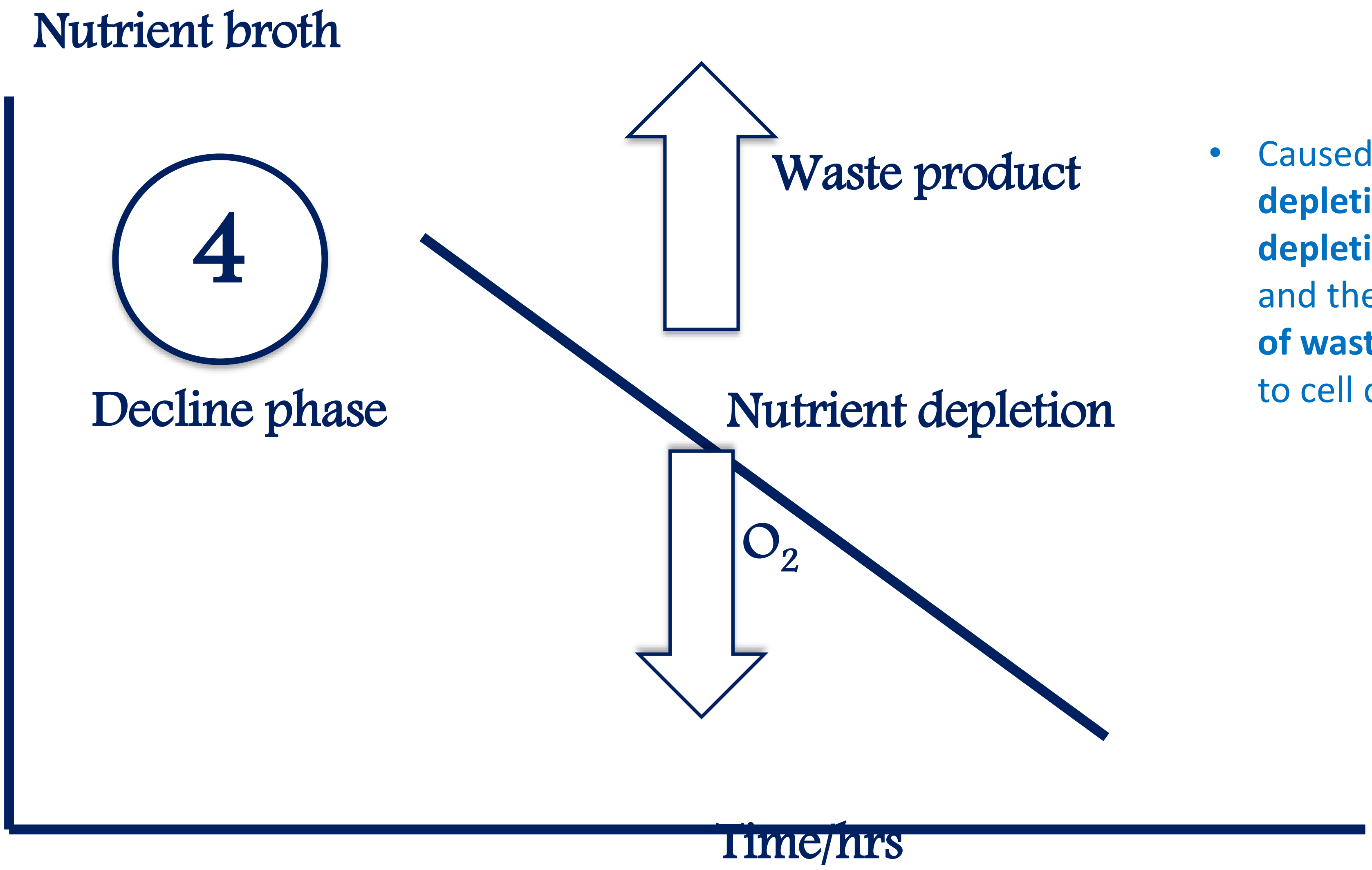


Constant number
No. of division = No. of death
(Waste product)

This state is caused by:

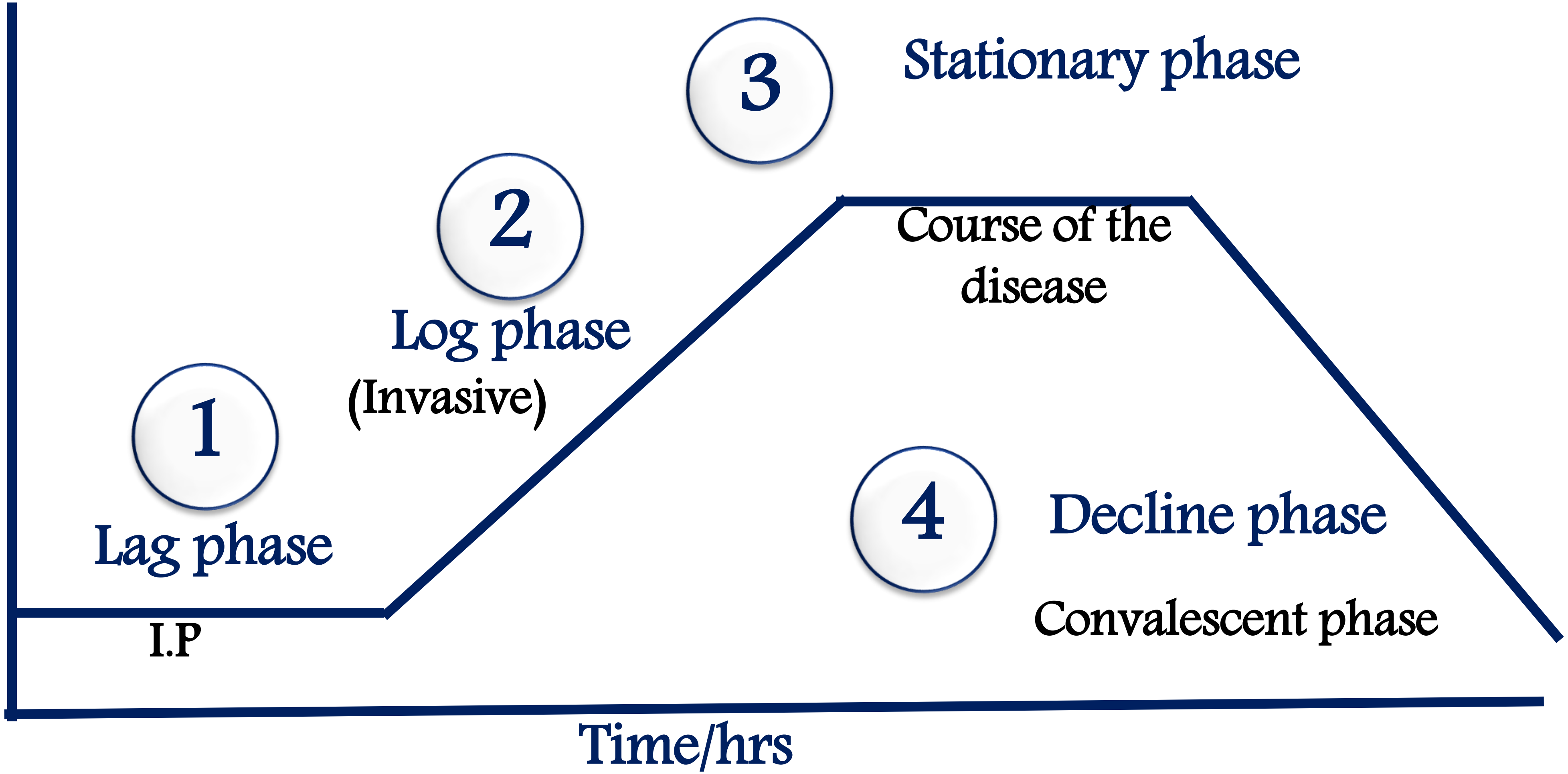
- Depletion of nutrients.
- Accumulation of waste products.

Decline phase



- Caused by the **severe depletion of nutrients**, **depletion of oxygen** (O2), and the **high accumulation of waste products**, leading to cell death.

Bacterial growth curve



The *in vitro* bacterial growth curve can be correlated to the stages of a bacterial infection in a human host:

- **Lag Phase** (in vitro) corresponds to the **Incubation Period (I.P.)** (in vivo), where the bacteria are adapting to the host.

- **Log Phase** (in vitro) corresponds to the **Invasive Phase** (in vivo), where bacteria multiply rapidly, and signs and symptoms of the disease appear.

- **Stationary Phase** (in vitro) corresponds to the **Course of the Disease** (in vivo), representing the peak of the illness.

- **Decline Phase** (in vitro) corresponds to the **Convalescent Phase** (in vivo), or the recovery stage, as the host's immune system or treatment overcomes the infection.

Bacterial growth requirements

Growth Requirements

A) Nutrition
B) Gaseous
C) Temp. & pH

A) Nutrition

Maintenance of bacterial growth

A) Nutrition

1- Autotrophic

auto = self
Trophic=nutrition

2- Heterotrophic

hetero = different
Trophic = nutrition

Autotrophic

Utilize simple inorganic substance

CO_2 → As a source of → Carbon

Ammonium → As a source of → Nitrogen

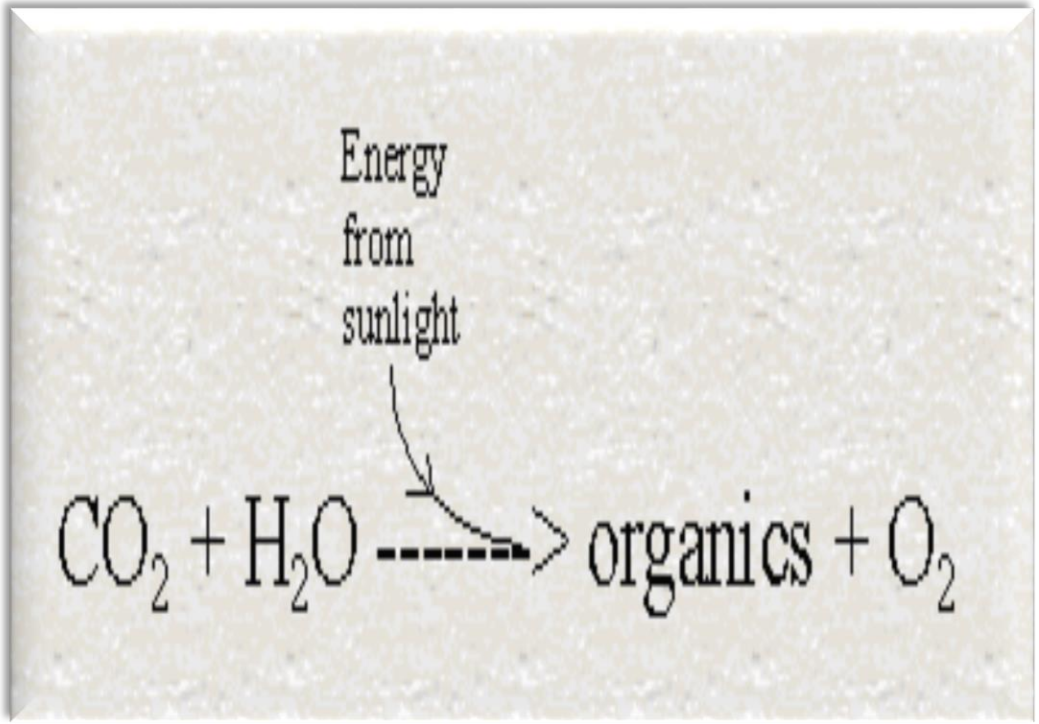
Carbon and Nitrogen → Complex organic materials

(Saprophytic)

(decaying), found in soil and air, and make their own food.

Autotrophic

No medical importance



- Because they are self-sufficient, they have no medical importance and do not cause disease.

Heterotrophic

Parasitic

These bacteria require complex preformed organic substances e.g. sugars, proteins etc.

Living host

- They must obtain these nutrients from a living host, meaning they are **parasitic**.
- Because they must infect a host to get nutrition, these bacteria are of medical importance.

Medical important

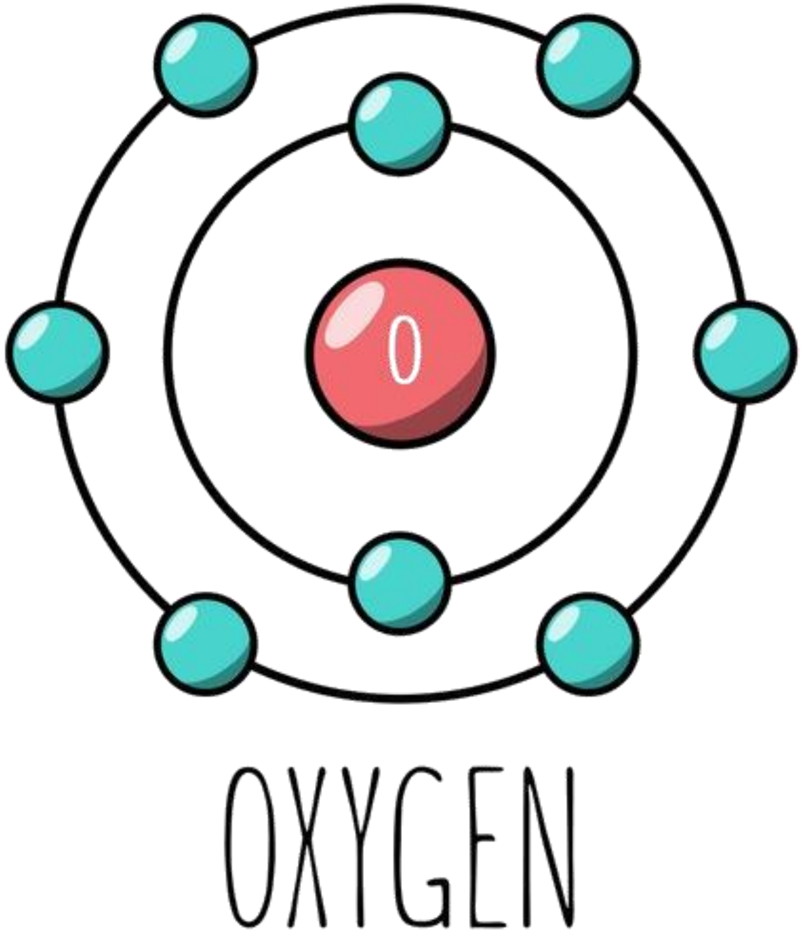
Growth Requirements

Gaseous requirements

O_2 requirement, bacteria are classified into 5 groups

O2 requirement

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



Respiration

- **Respiration** in bacteria does *not* mean breathing (inhalation/exhalation). It refers to **glucose catabolism** for the purpose of **energy production (ATP)**.

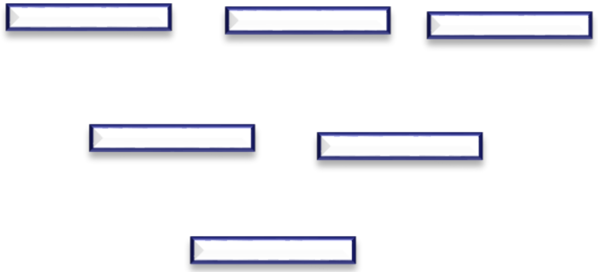
✓ **Aerobic Respiration:** Energy production that **requires oxygen**.

✓ **Anaerobic Respiration:** Energy production that **does not require oxygen**.

1- Obligate aerobes (Aerobic respiration)

Presence of O_2

Absence of O_2



Growth

No growth

e.g. Pseudomonas aeruginosa

1- Obligate aerobes (Aerobic respiration)

Aerobic respiration

⇩

For production Energy (ATP)

⇩

By Glucose catabolism (glycolysis)

1- Obligate aerobes (Aerobic respiration)

Glucose +ADP → 2 pyruvate + 2 ATP

$2NAD^+$ → 2NADH

2 pyruvate —Kreb's Cycle→ $2FADH_2$ + 8NADH + 2 ATP

Oxidative phosphorylation

O_2 → Carry $2H^+$ → H_2O + 34 ATP

38 ATP

Metabolism (Aerobic Respiration):

1. **Glycolysis:** Glucose is converted to 2 Pyruvate, generating **2 ATP**.

2. **Krebs Cycle:** The 2 Pyruvate enters the Krebs cycle, generating **2 ATP**.

3. **Oxidative Phosphorylation:** In the presence of **oxygen**, which acts as the final electron/hydrogen carrier, this pathway generates a large amount (**34 ATP**).

4. **Total Yield: 38 ATP**. This high energy yield helps the bacteria survive and reproduce efficiently.

1- Obligate aerobes

Highly toxic molecules

Superoxide (O_2) — (H_2O_2)

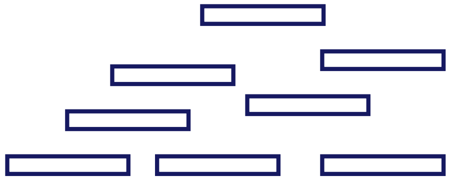
↑ Superoxide dismutase — ↑ Catalase

- **Toxic Byproducts:** This process creates highly toxic molecules (reactive oxygen species) like superoxide (O2) and hydrogen peroxide (H2O2).
- **Defense Mechanism**: Obligate aerobes possess two critical enzymes to neutralize these toxins:
  - **Superoxide dismutase (SOD):** Breaks down superoxide.
  - **Catalase:** Breaks down hydrogen peroxide.

2- Obligate anaerobes

Presence of O2

Absence of O2



No growth

Growth

Bacteroides fragilis

2- Obligate anaerobes(Anaerobic respiration)

Glucose +ADP → 2 pyruvate + 2 ATP
(2NAD+ → 2NADH)

2 pyruvate —Kreb's Cycle→ $2FADH_2$ +8NADH + 2 ATP

4 ATP

✗ O_2 ⇨ Other pathway

Lack Superoxide dismutase &Catalase

2- Obligate anaerobes(Anaerobic respiration)

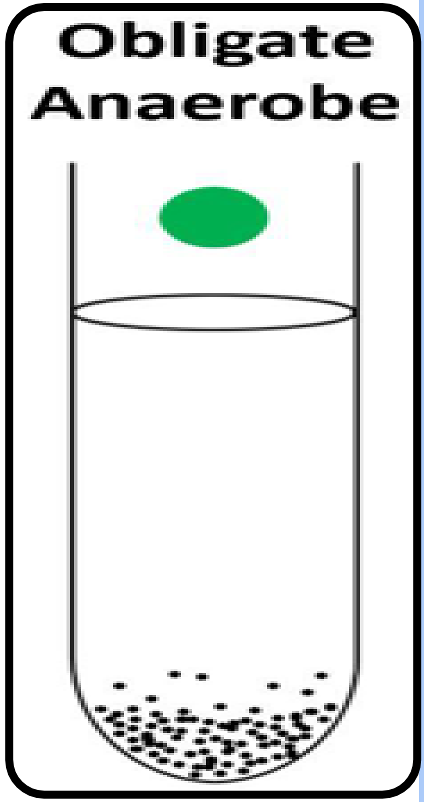
The organism used inorganic molecules

Nitrate
Sulfate
Co2

Carry H^+

13 ATP + 4 ATP
17 ATP

Lack
Superoxide dismutase
Catalase



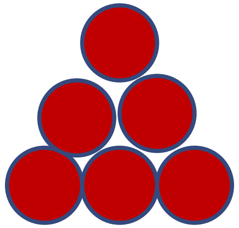
Metabolism (Anaerobic Respiration):

1. **Glycolysis**: Glucose is converted to 2 Pyruvate, generating 2 ATP.
2. **Krebs Cycle**: The 2 Pyruvate enters the Krebs cycle, generating 2 ATP.
3. **Total Yield (Pathway 1):** 4 ATP.
4. **Alternative Pathway:** Since O2 is not available to carry the hydrogen/electrons, the organism uses inorganic molecules (like Nitrate, Sulfate, or CO2) as the final carrier. This pathway generates an additional 13 ATP.
5. **Total Yield (Pathway 2):** 17 ATP (4 + 13).

- **Defense Mechanism**: Obligate anaerobes **lack** superoxide dismutase (SOD) and catalase.
- **This is why oxygen is toxic to them**: if O2 is present, toxic byproducts are formed, but the bacteria have no enzymes to neutralize them, and they die.

3- Facultative anaerobes

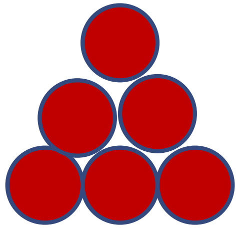
Presence of O_2



Growth

Rate of growth

Absence of O_2



Growth

Most bacteria

They are primarily aerobic, so their rate of growth is **better** in the **presence** of O2, but they can live without it.

3- Facultative anaerobes

Anaerobes

Fermentation

Glucose glycolysis 2 Pyruvate + 2ATP

No Kreb's cycle & other pathway

(Absence of carriers)

Acid & Alcohol

Metabolism:

In Aerobic conditions (Presence of O_2):

- They perform **full** aerobic respiration (Glycolysis, Krebs, Oxidative Phosphorylation) and produce **38 ATP.**

In Anaerobic conditions (Absence of O_2):

- They perform **Glycolysis** (Glucose -> 2 Pyruvate), **generating 2 ATP.**
- They cannot enter the Krebs cycle (lacking the O_2 carrier) or the other anaerobic pathway (not used to nitrate/sulfate).
- The pyruvate is instead broken down into acid and alcohol. This process is called **Fermentation**.
- This allows them to survive anaerobically, but it is much less efficient (**only 2 ATP**).

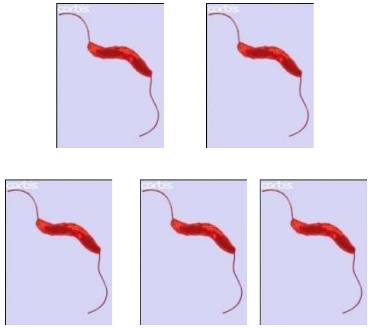
4- Micro-aerophilic

Presence of O_2

superoxide & H_2O_2

No growth

Low O_2



2-10% O_2

Growth

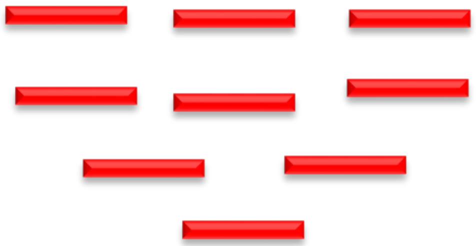
Low superoxide dismutase & catalase

Campylobacter
Helicobacter

- Micro-aerophilic bacteria ("micro" = small) cannot grow in the presence of normal atmospheric O_2.
- They require a low percentage of O_2 (e.g., 2-10%) to grow.
- **Examples**: Campylobacter, Helicobacter.
- **Defense Mechanism**: They possess low levels of superoxide dismutase (SOD) and catalase.
  - These low enzyme levels are sufficient to neutralize the small amount of toxic byproducts (superoxide, H_2O_2) produced by the low O_2 concentration.
  - They cannot survive in normal O_2 levels because the large amount of toxic byproducts would overwhelm their limited enzyme supply.

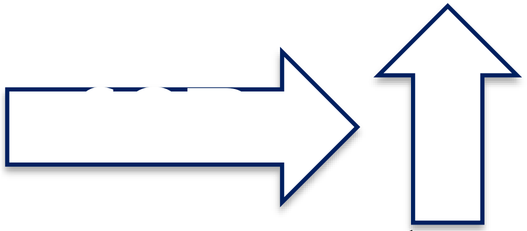
5- Aero-tolerant anaerobes

Low O2



Superoxide dismutase

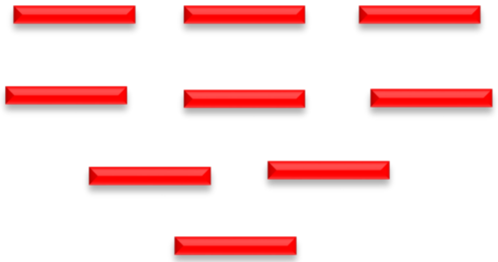
O_2



H_2O_2

Tolerate

Absence of O2



Growth

Cl.perfringens

- **Aero-tolerant Anaerobes** are primarily anaerobic (their origin growth is in the absence of O2).
- However, they can "**tolerate**" or **withstand** the presence of low oxygen.
- **Example**: *Clostridium perfringens*.
- **Defense Mechanism:**
  - They **possess superoxide dismutase (SOD),** which allows them to withstand **some** oxygen.
  - **They lack catalase**. Therefore, if H_2O_2 (hydrogen peroxide) accumulates, they cannot neutralize it and will die. They prefer anaerobic conditions.

Growth Requirement: CO2 requirements

CO_2 (0.03%)

Present in air is sufficient

CO_2 (5–10%)

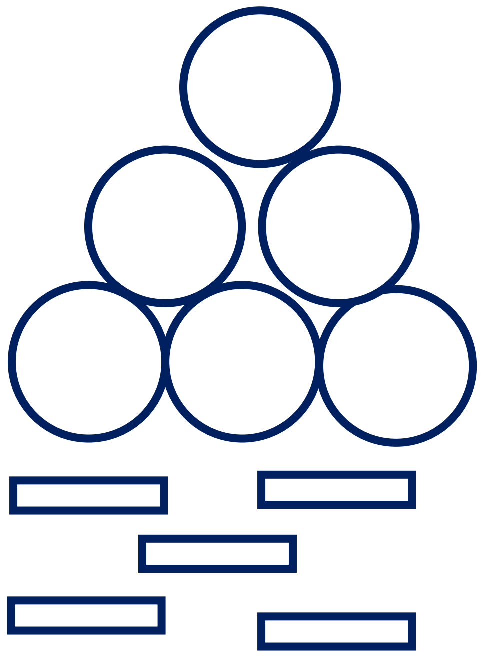
(Capnophilic)

Neisseria
Brucella

- **The normal percentage** of CO2 (Carbon Dioxide) in the air (0.03%) is sufficient for most bacteria.
- However, some bacteria require a **much higher concentration** of CO2 (5-10%) to grow.
  - ✓ These bacteria are called **capnophiles**.

Growth Requirement: Hydrogen ion (pH)

pH (7.2 – 7.4)
(Most bacteria)

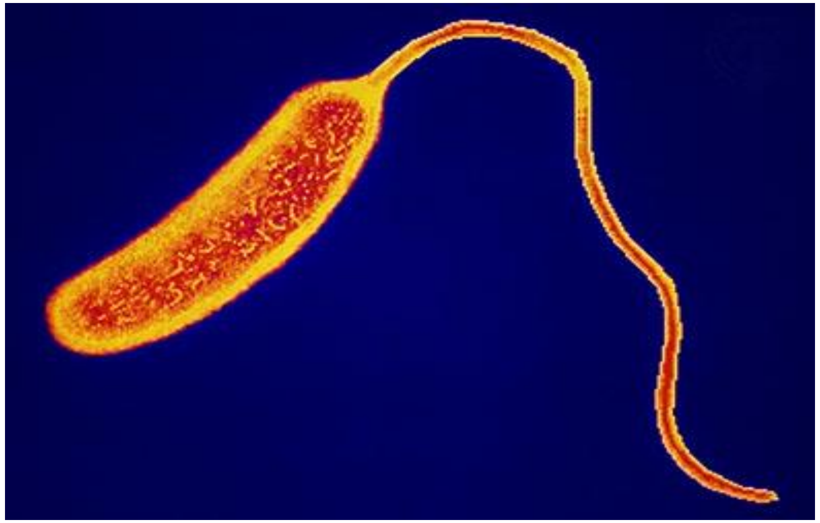


- **Neutrophils**: Most bacteria prefer a **neutral pH**, typically between 7.2 and 7.4.

Hydrogen ion (pH)

Alkaline (pH 9)

Alkaliphiles



Vibrio cholerae

Acidic (pH 4)

Acidophiles

Lactobacilli

Growth Requirement: Temperature

Mesophilic
(20-45 c)
(Most bacteria)

Psychrophilic
(0-15 c)

Thermophilic
(55-65 c)

GENOME

جينوم

شاركنا تعديلاتك للموديفايد عبر هذا الرابط :

اضغط هنا

Version	Slide No and place of error	Before Correction	After Correction

"خيرُ الناس أنفعهم للناس"