MICROBIOLOGY

بسم الله الرحمن الرحيم

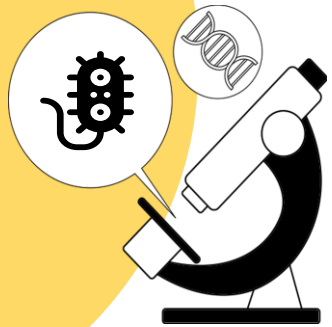
MID – Lecture 8

Bacterial Growth & Physiology

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

- Written by:
- **Mohammad Shwyiat**
- **Ahmad Abu Aisha**
- Reviewed by:
- **Laith Joudeh**



Lecture 3

Bacterial Growth& physiology

Bacterial Growth

Definitions

B. Reproduction

culture media

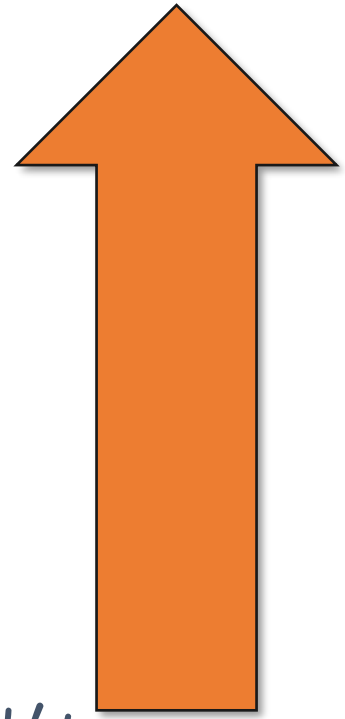
A) Definition

B) Classification

C) Types

Bacterial growth curve

Bacterial Growth: Definition

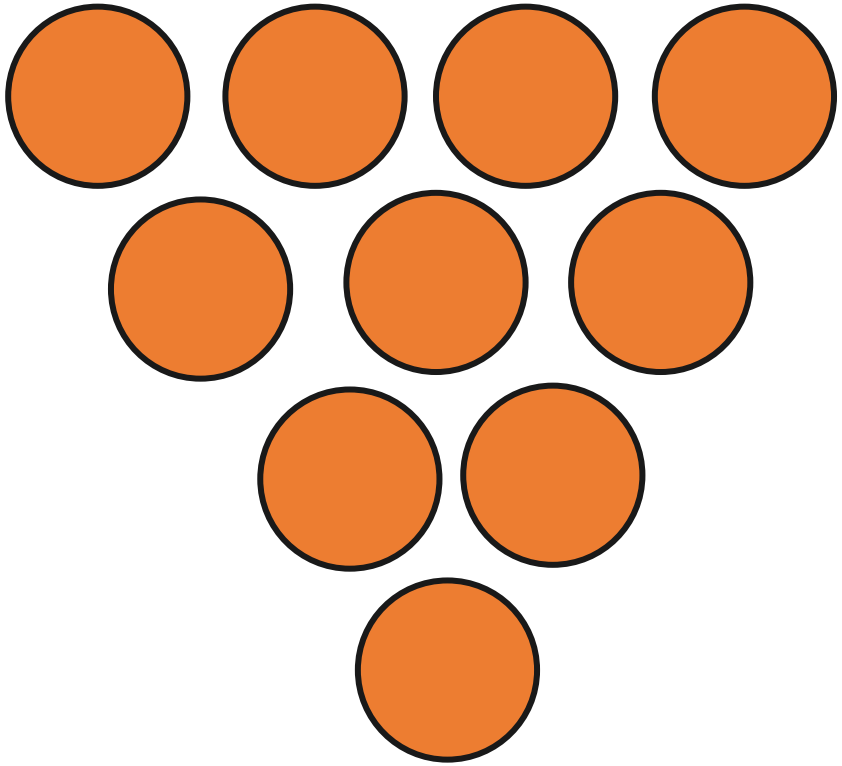


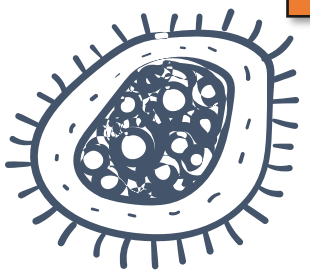
Increase in

Size

&Number of

organism



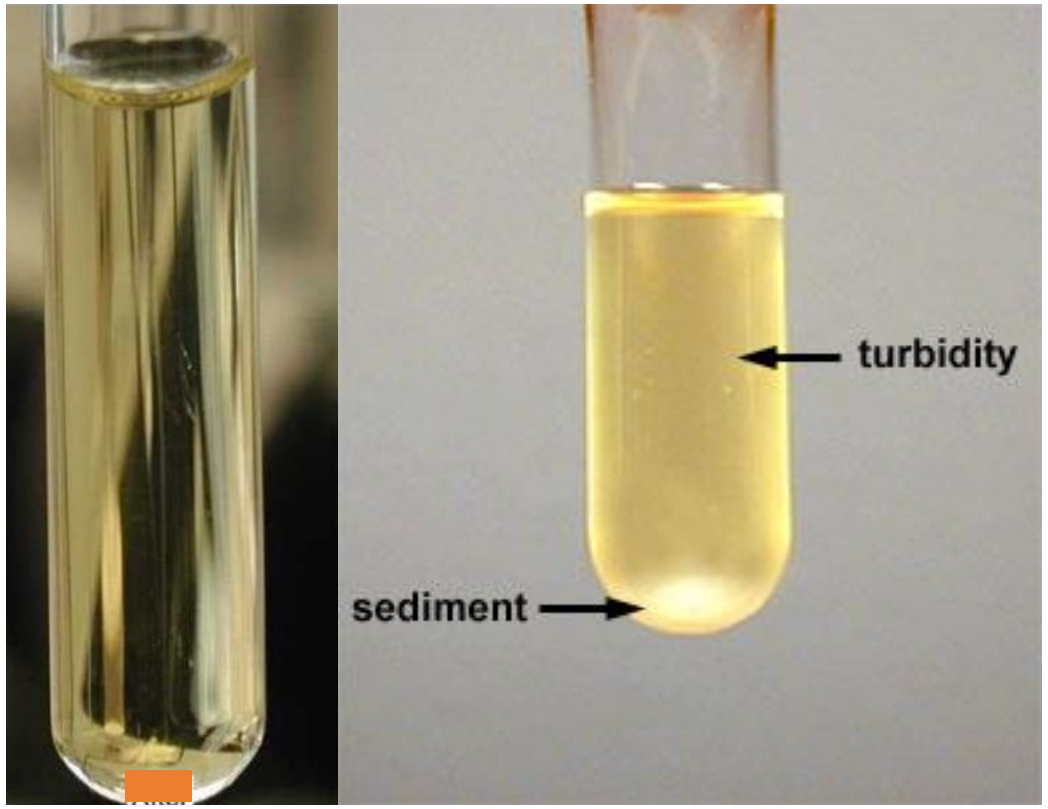


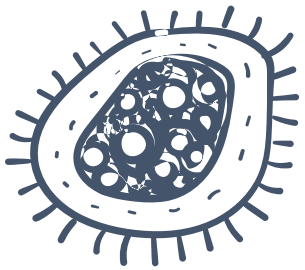
1) Bacterial Growth

We put the sample in a broth (fluid media) then leave it for hours. An increase in Turbidity (cloudiness) indicates bacterial growth.

Indicated by

a) Turbidity of the fluid media



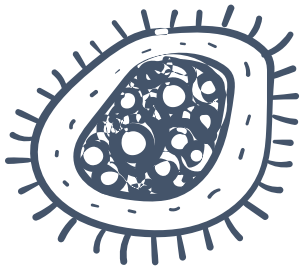


1) Bacterial Growth

Indicated by

b) Colonies on solid media

(Macroscopic product)



We place the sample on a solid medium, where colonies visible to the naked eye (macroscopic) indicate bacterial growth.

2) Colony (Macroscopic product)

Single bacterium

On solid media

After 20-30 division

Binary fission

Colony

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

A colony is a product of a single bacterium after it undergoes 20-30 binary fissions.

2) Colony

After 20-30 division

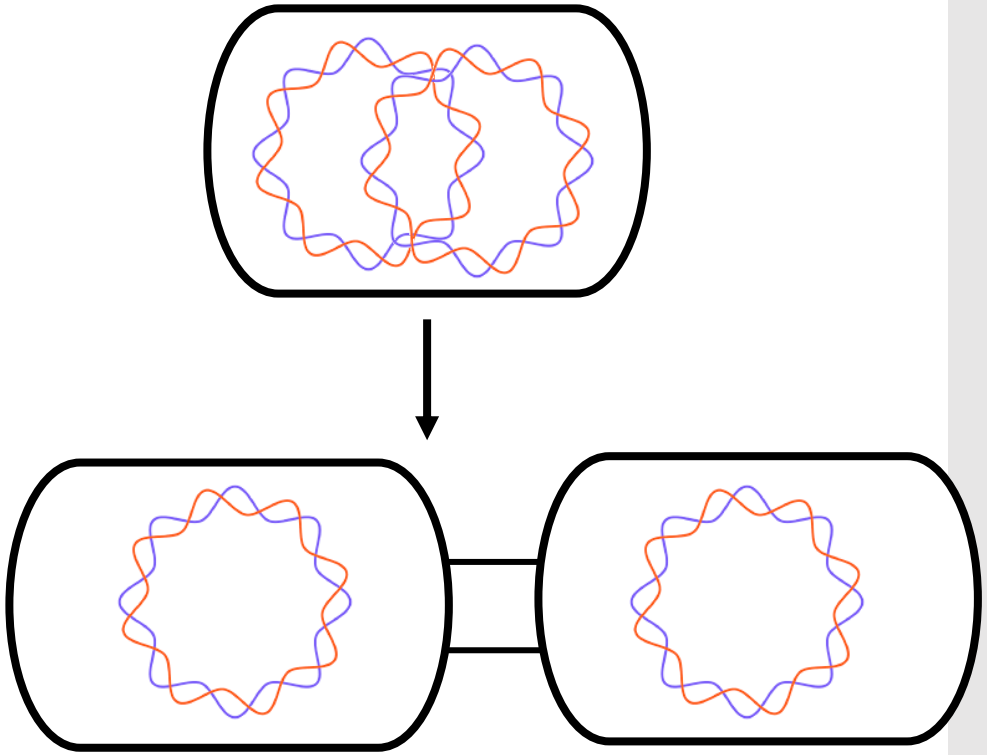
Binary fission

1 Million

1 million and 24 thousand bacteria result

(2^{20}) 20 is the number of divisions

The first division results in 2 daughter cells, hence the base is 2

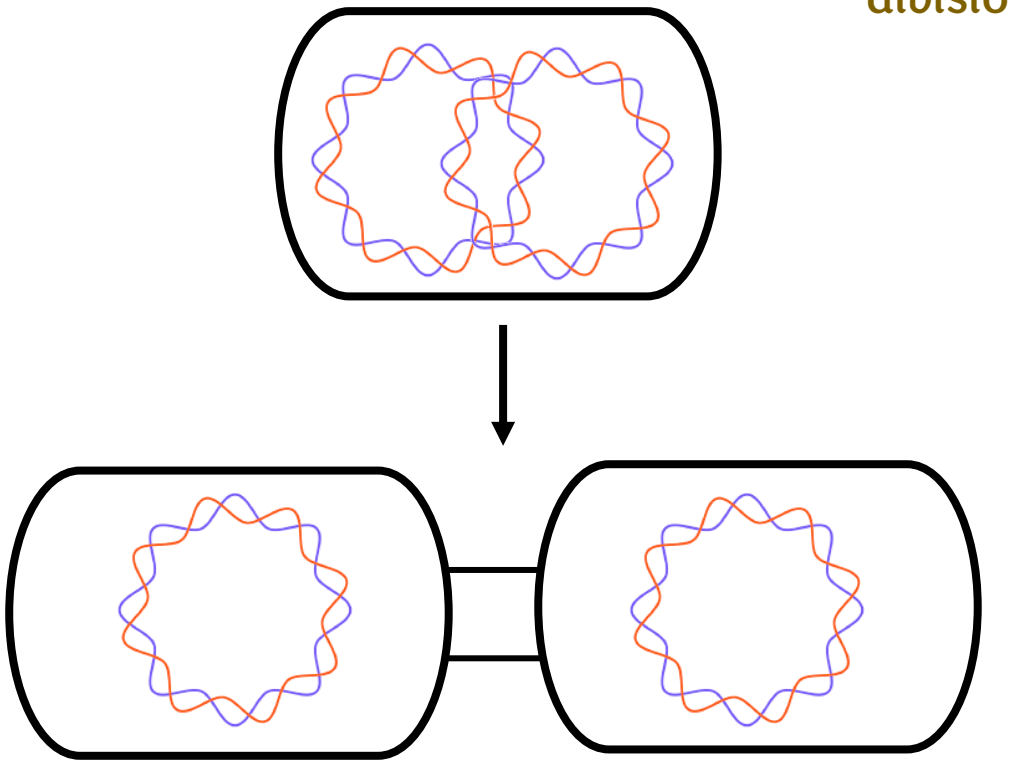


3) Generation time (doubling time)

Generation time = time it takes the bacteria to undergo division

13min (*V. cholerae*)

V. cholerae undergoes division every 13 minutes.



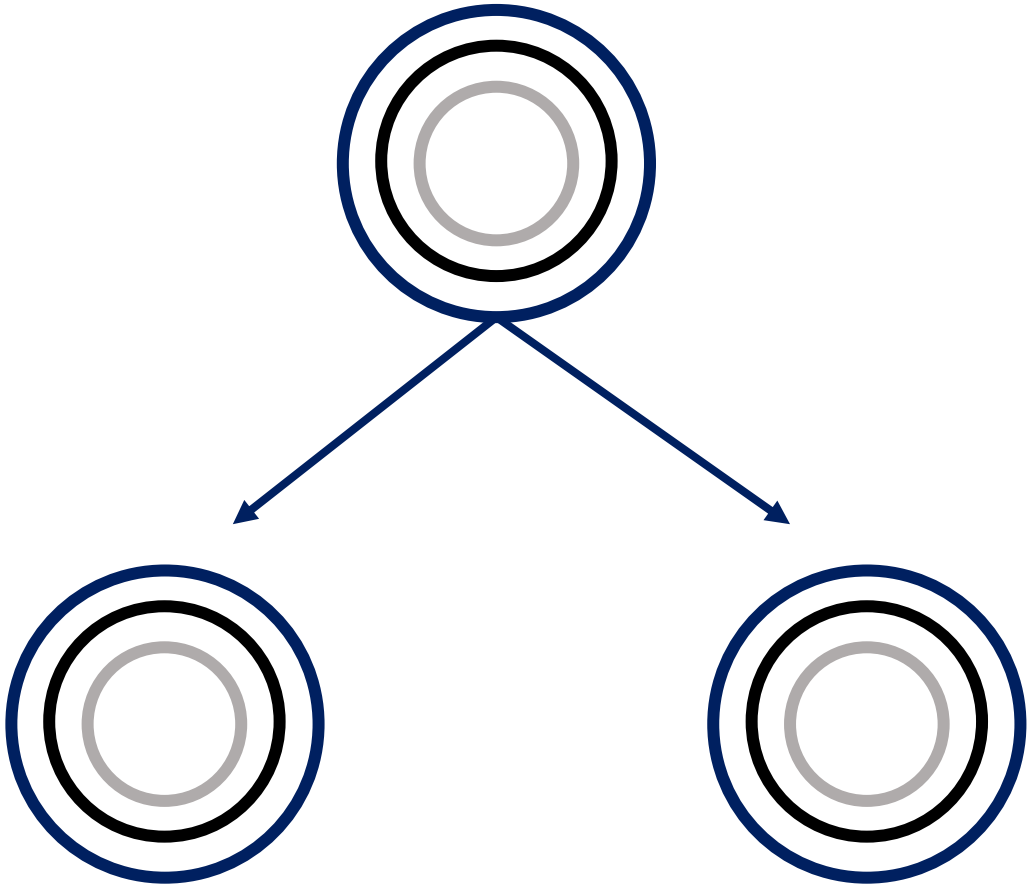
24 hrs (*M. tuberculosis*)

M. tuberculosis undergoes division every 24 hours

Bacterial Reproduction

Binary fission

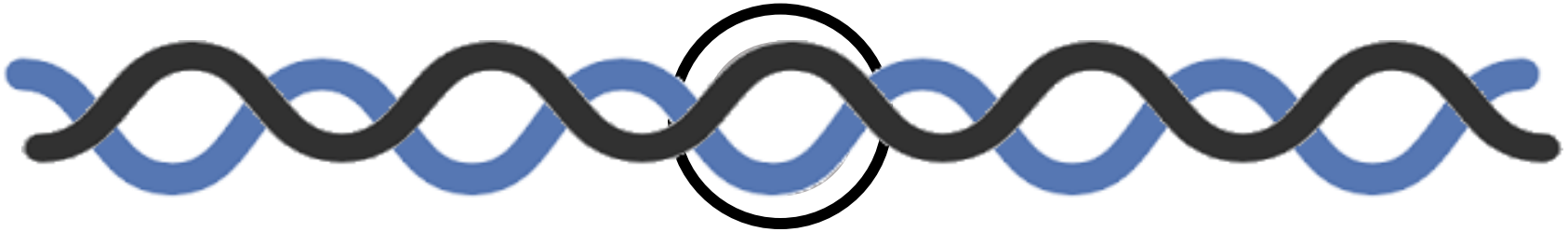
One cell produces 2 genetically identical daughter cells.



Bacterial Reproduction

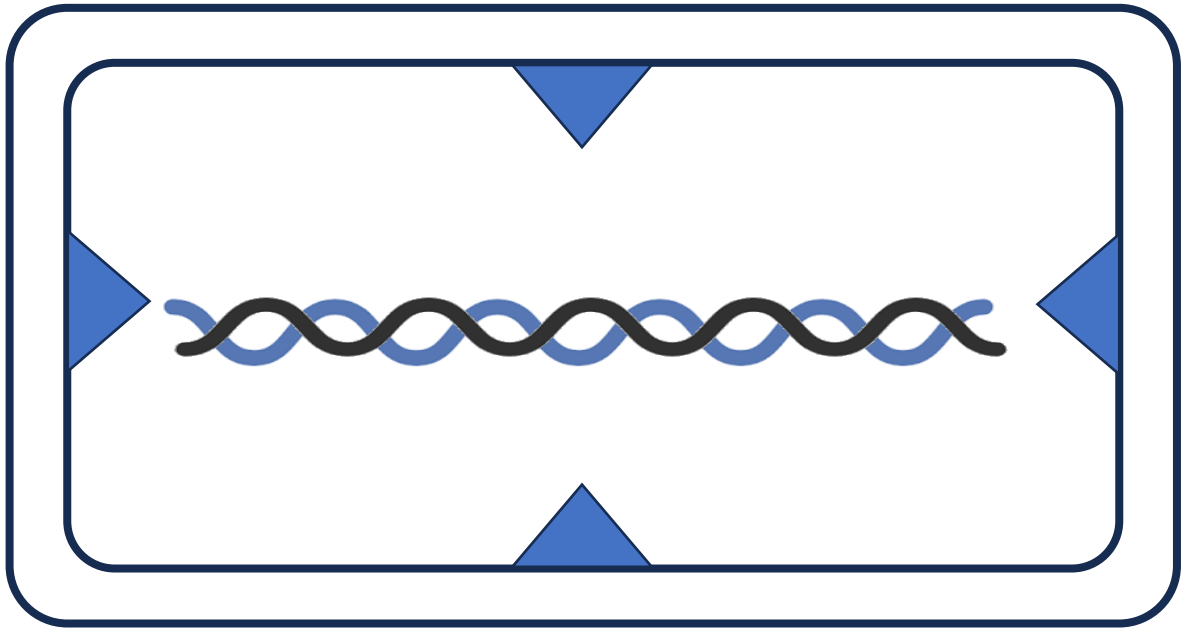
1 Elongation Of the DNA

Elongation happens in single direction only.



Bacterial Reproduction

2 **Separation of 2 strands (ssDNA attached to mesosome)**

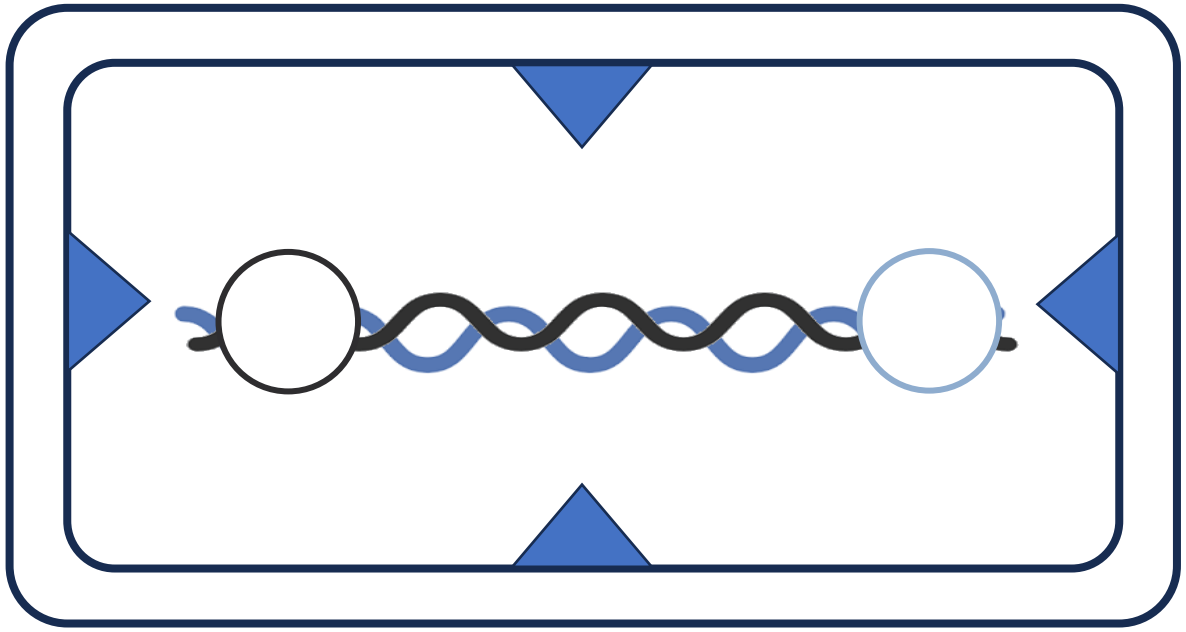


Every single strand will get attached to the mesosome
This occurs because enzymatic activity takes place there, where enzymes have the ability to separate the double-stranded DNA.

Bacterial Reproduction

2 **Separation of 2 strands**

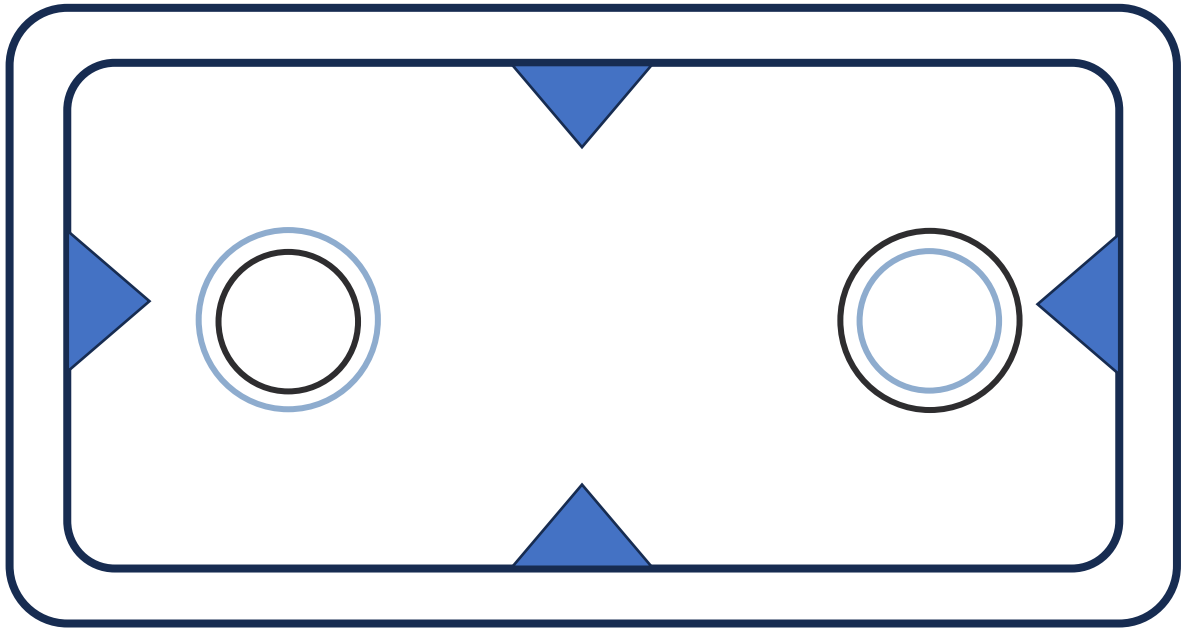
Each strand returns to its original state (circular)



Bacterial Reproduction

3 Separate ssDNA & become dsDNA

Each strand will act as a template, building a double strand



Bacterial Reproduction

4

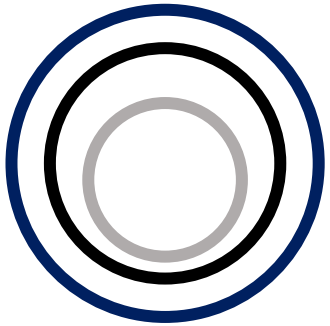
Formation of division septum

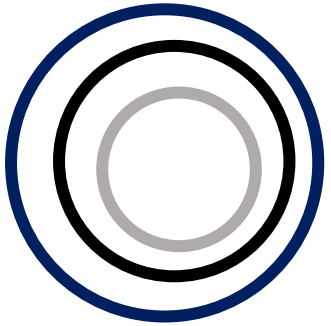
In bacteria, cell division occurs by the ingrowth of the envelope layers [membrane and peptidoglycan (PG) cell wall] to form a septum that. The septal PG is later hydrolyzed, and the daughter cells separate. [1]

Bacterial Reproduction

5

Cell separation





Finally, the cell separates, producing 2 daughter identical cells

Bacterial culture media

Bacteria grow

(In vitro)

In vitro means "in glass," referring to an artificial setting in which nutrients are provided.

Need nutrients for growth

Artificial

Purpose

Of culture media

Is to ultimately determine whether the patient suffers from a bacterial infection or not.

1

Study Properties

Of the bacteria

Purpose

2

Isolation & diagnosis (Causative agent)

Determining the type of bacteria which caused the infection

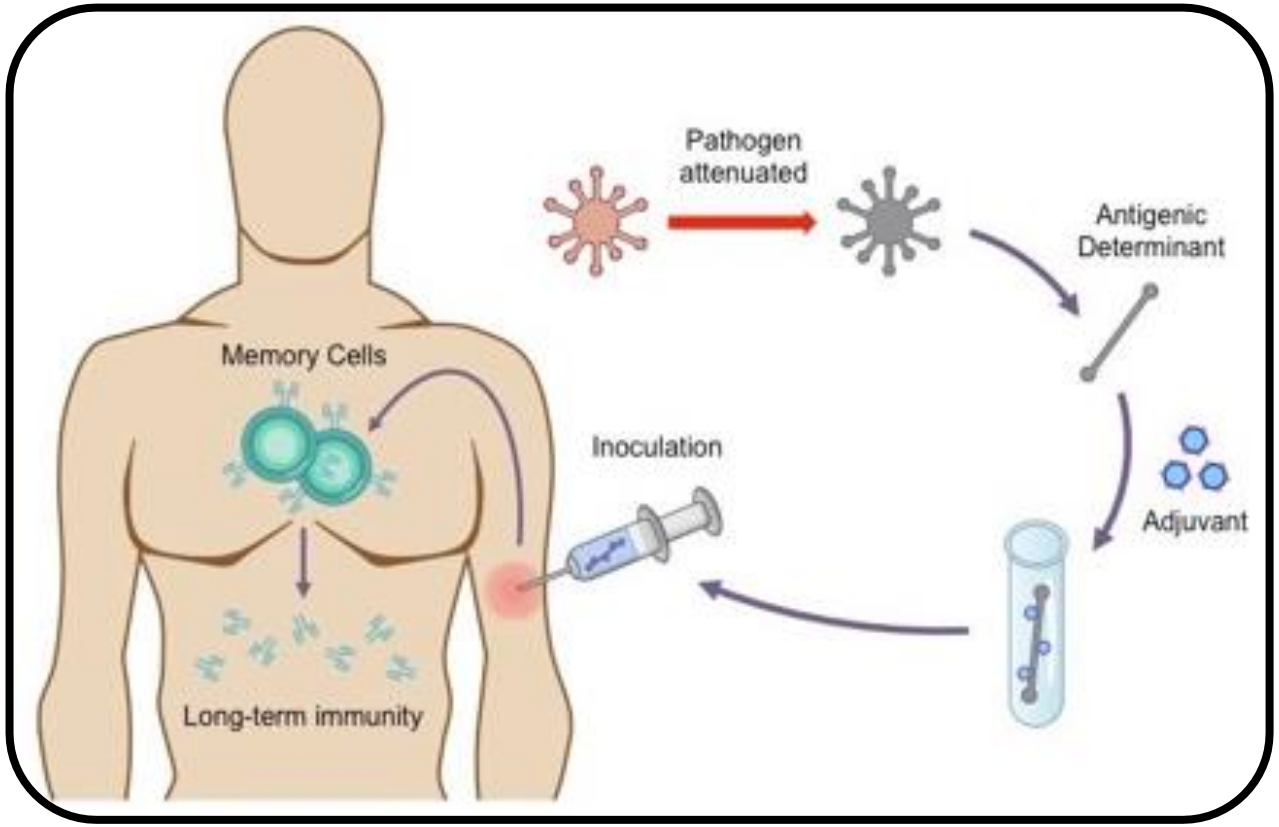
Purpose

3

Prepare vaccine &

Other products

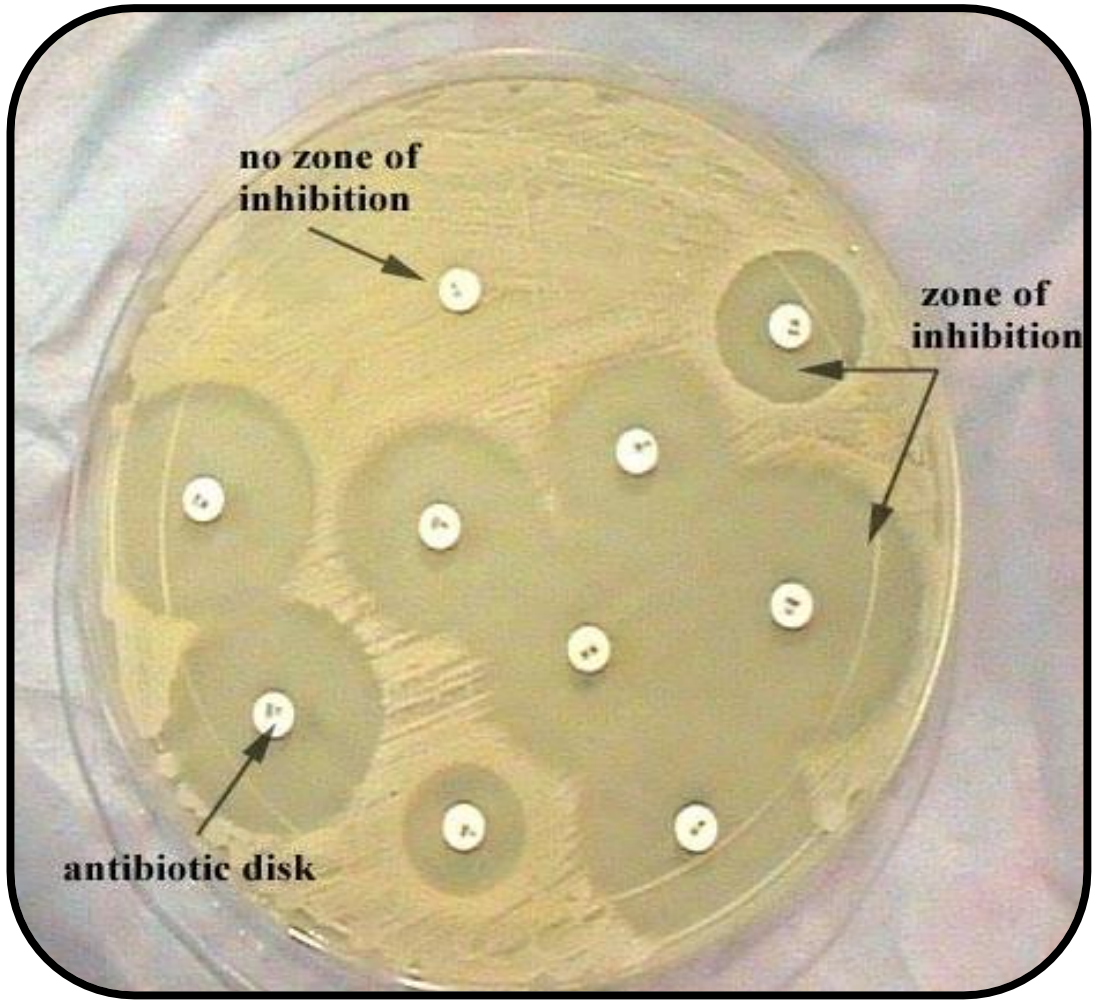
We use *S. Pyogenes* as an anticoagulant.



Purpose

4

For selecting proper antibiotics



Classification of media

Liquid

We put the media in a tube if it's liquid and call it broth.

Solid

Is put in a petri dish

Types of media

1) Simple media

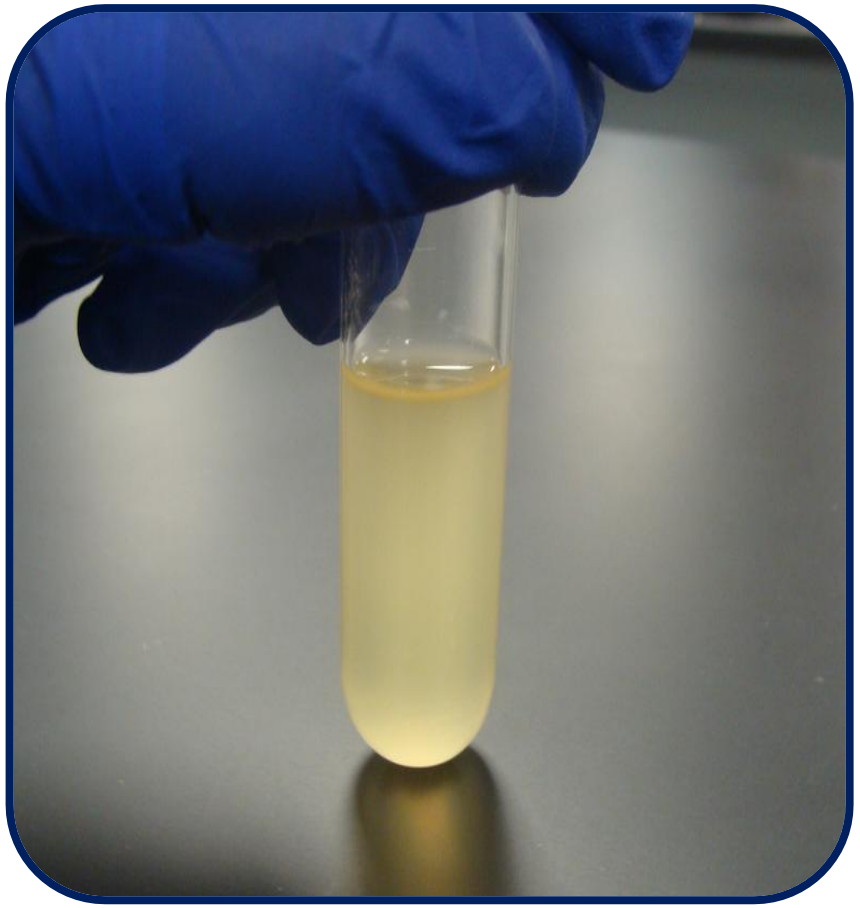
2) Enriched media

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 the growth

Sugar media

Simple media

B) Nutrient broth

Meat extract

Enhancement

Simple media

C) Nutrient agar plate

Nutrient broth + 2%

agar agar

(Seaweed)

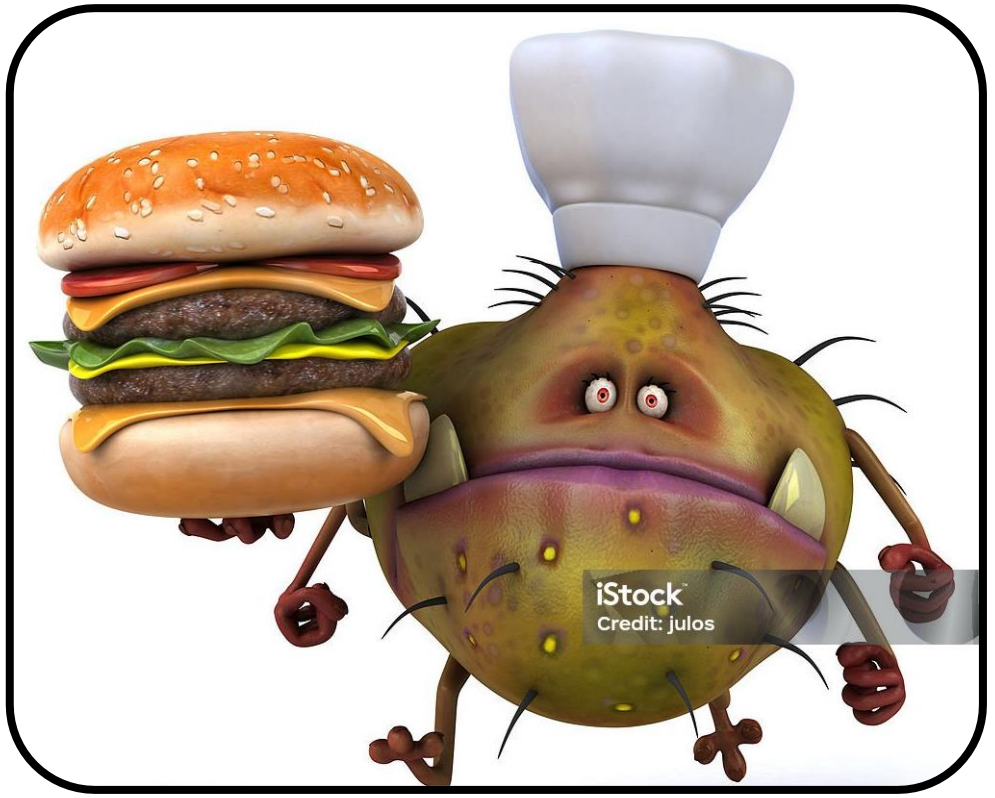
Boiled together and left at room temperature till solidification.

Suitable for ***Staph. aureus***

Enriched media

Fastidious bacteria

Need blood, serum for growth



Fastidious bacteria require more than just proteins; they require other nutrients, such as blood and serum.

Enriched media

A) Blood agar

Nutrient agar heated at 45°C

(semisolid)

+ sheep blood

Sheep blood doesn't contain antibodies that can affect the result

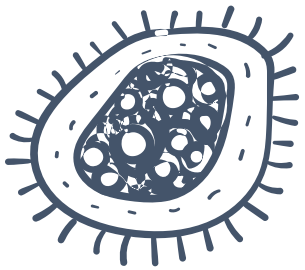
Enriched media

A) Blood agar

Streptococci

Strept. pyogenes

Streptococci mainly grows on this medium, some can exhibit hemolytic activity.



Hemolysis on blood agar:

A. Complete (beta) hemolysis:

1. *Staphylococcus aureus*
2. *Streptococcus pyogenes*

Complete and clear

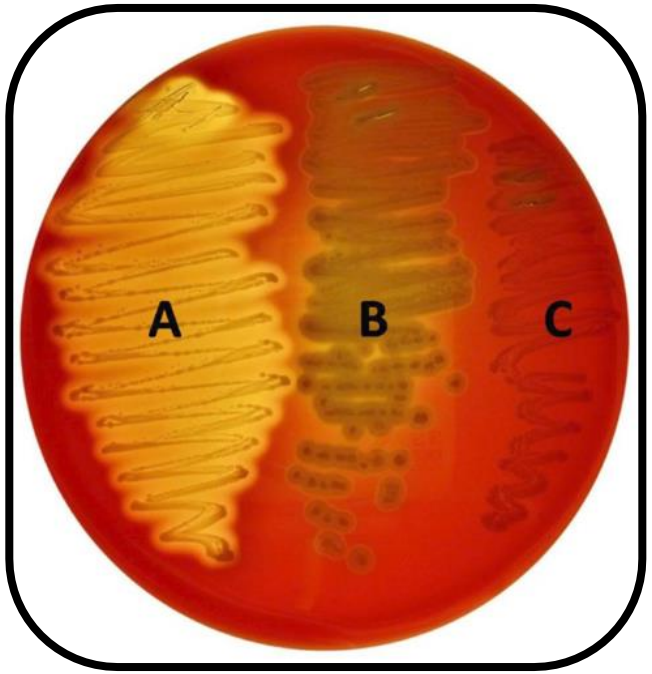
C. No (gamma) hemolysis:

- *Enterococci.*

B. Partial (alpha) hemolysis:

1. *Streptococcus viridans*
2. *pneumococci.*

Partial and greenish

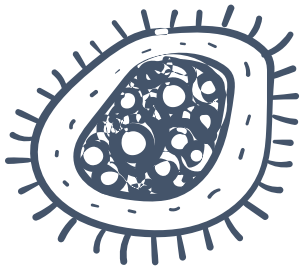


Enriched media

B) Chocolate agar

Nutrient agar heated at 100°C, add blood

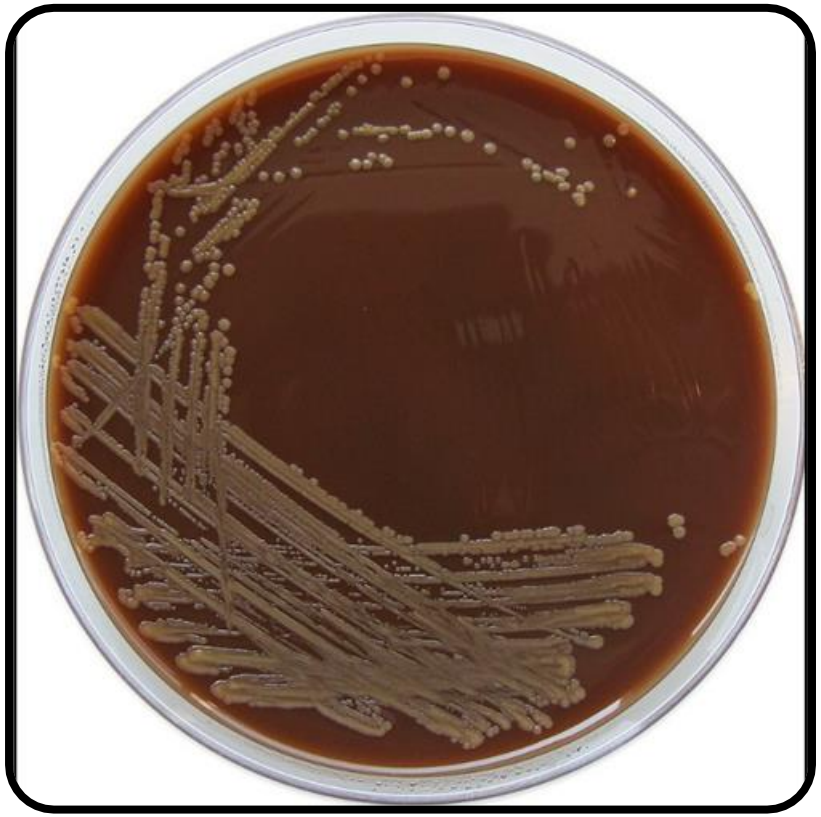
Hb —Heat→ **Haematin (Chocolate)**



Hemoglobin breaks down under heat, turning to haematin, which is brownish or chocolate colored.

Enriched media

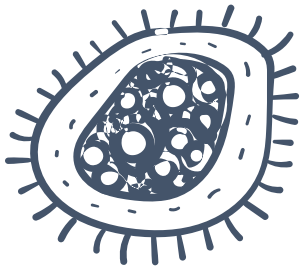
B) Chocolate agar



Haemophilus

Neisseria

Both are fastidious bacteria grown on chocolate agar



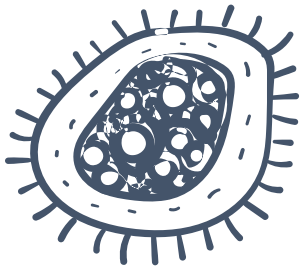
Selective media

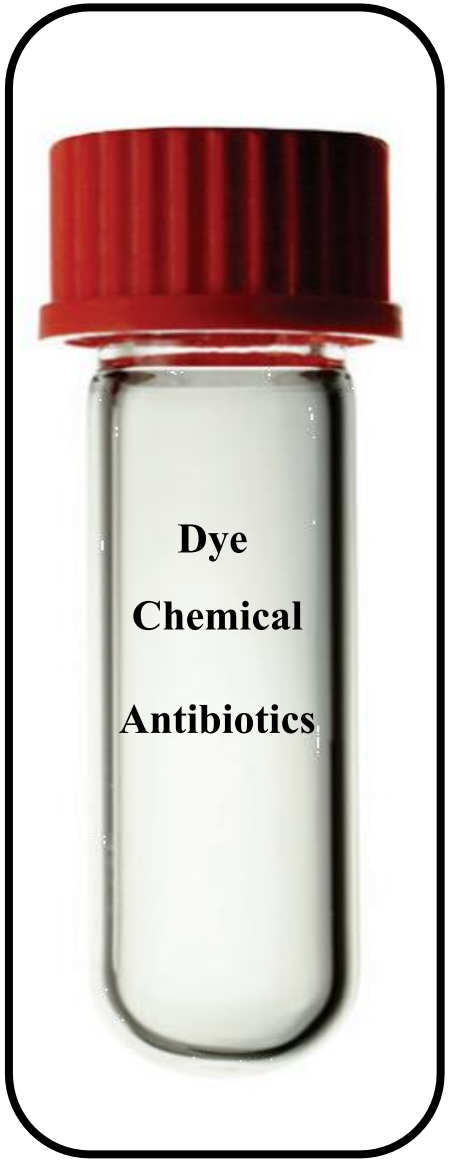
Allow a certain organism to grow

(Selective) &

inhibits the growth of others

Can support certain organisms and inhibit others, hence the name.





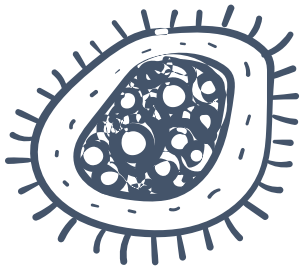
Selective media

Lowenstein Jensen medium

Malachite green The selective material

Mycobacterium tuberculosis

Is the bacteria grown In this medium



Selective media

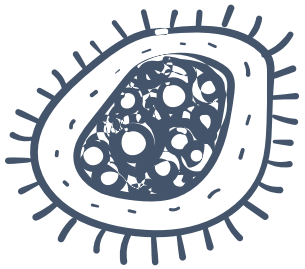
Blood tellurite agar

Potassium tellurite

The selective material

C.diphtheriae

The bacteria grown



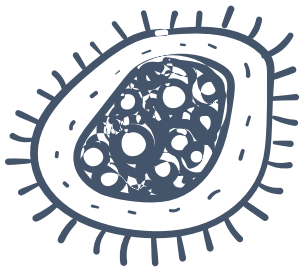
Differential media

The only difference between selective and differential medias

Selective + **Indicator**

Allow a certain organism to grow

Indicator to differentiate (change in visibility)



Purpose

Used to differentiate between bacteria that can ferment lactose and bacteria that can't

MacConkey's agar

1. **Bile (Enterobacteria)**

Bile is selective for enterobacteria

2. **Lactose = test sugar**

3. **Peptone**

Peptone: Nitrogen source, also works as a carbon source for bacteria that can not metabolize sugars. [2]

4. **Neutral red = pH indicator**

If the organism can ferment lactose ➔Pink because fermentation of lactose produces acid

If not➔ pale "yellowish"

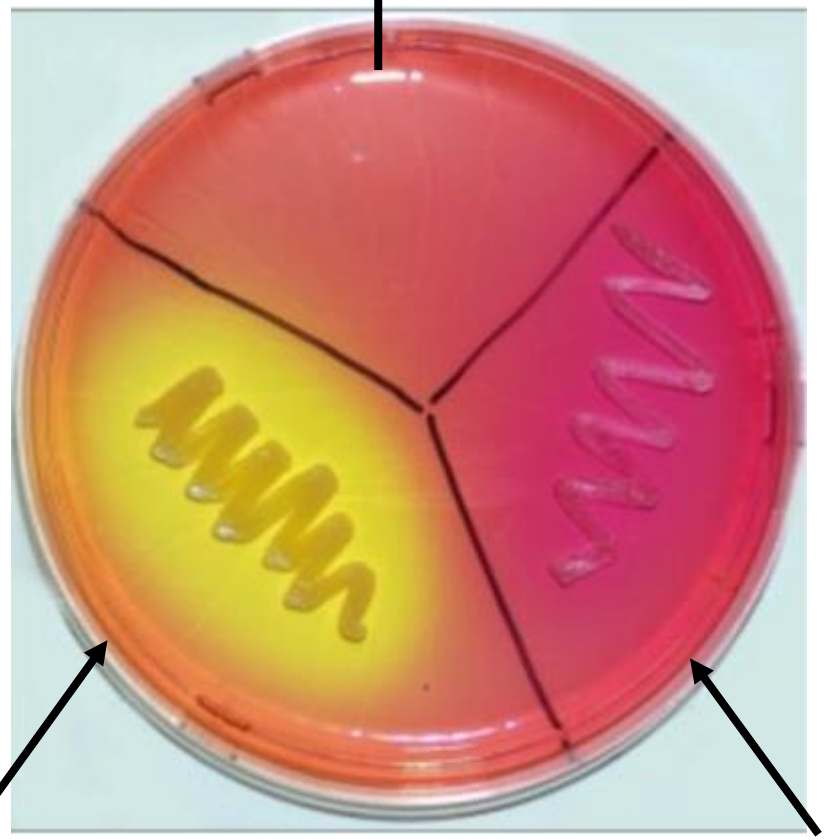
Pink **Pale**

Differential media

Mannitol salt agar

(high salt 7.5% NaCl)

Phenol red = pH indicator



Can ferment Mannitol ➔ acid

Differential media

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

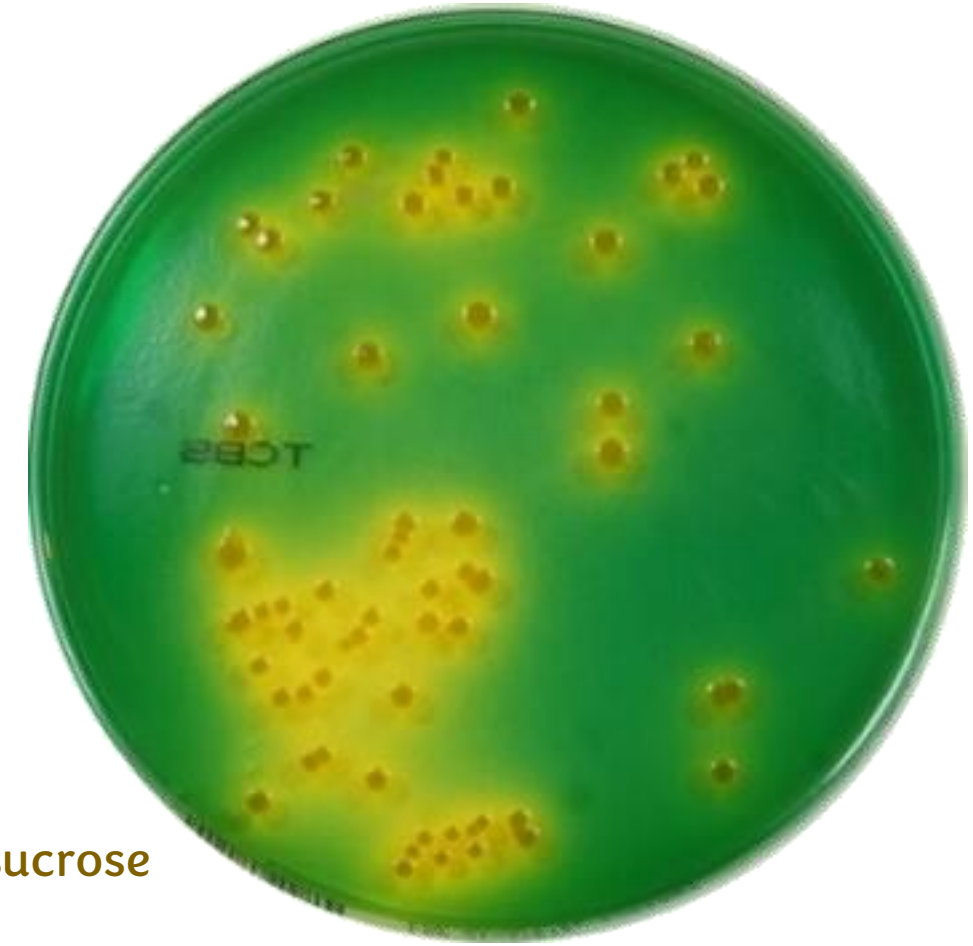
Thiosulphate

Bile

Citrate

Sucrose as test sugar

Bromothymol blue Indicator



Vibrio cholera appears yellow, because it can ferment sucrose
Vibrio parhaemolyticus can't ferment sucrose ➔ green

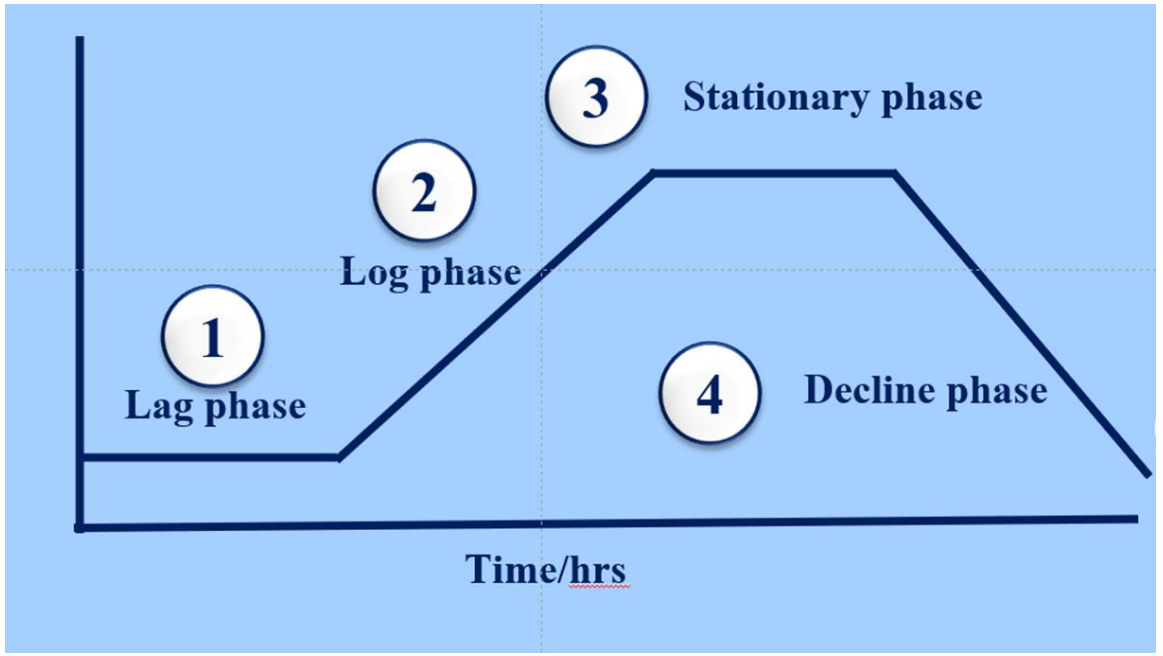
Bacterial growth curve

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

Bacterial growth curve

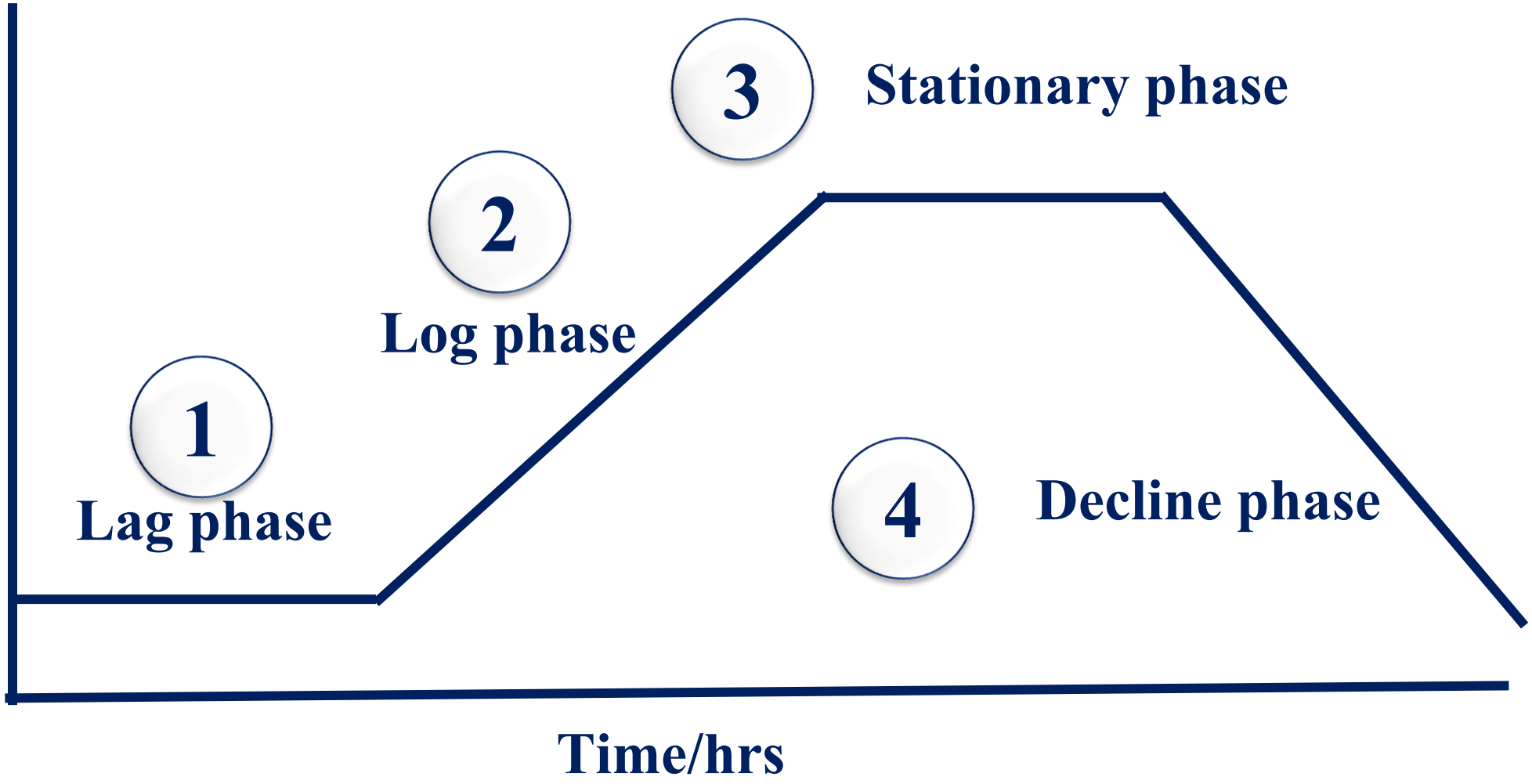
If a small number of bacteria are inoculated into a liquid nutrient medium

They experience 4 phases



72h

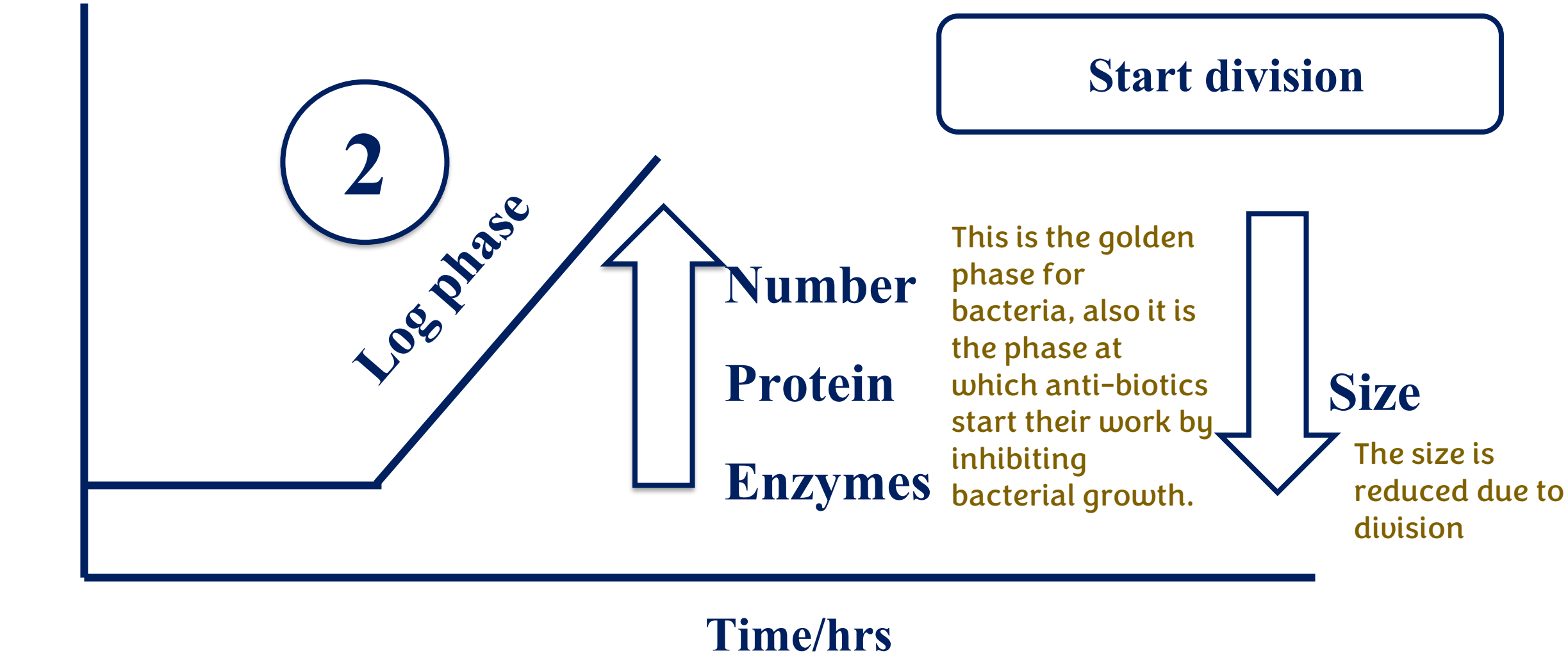
Bacterial growth curve



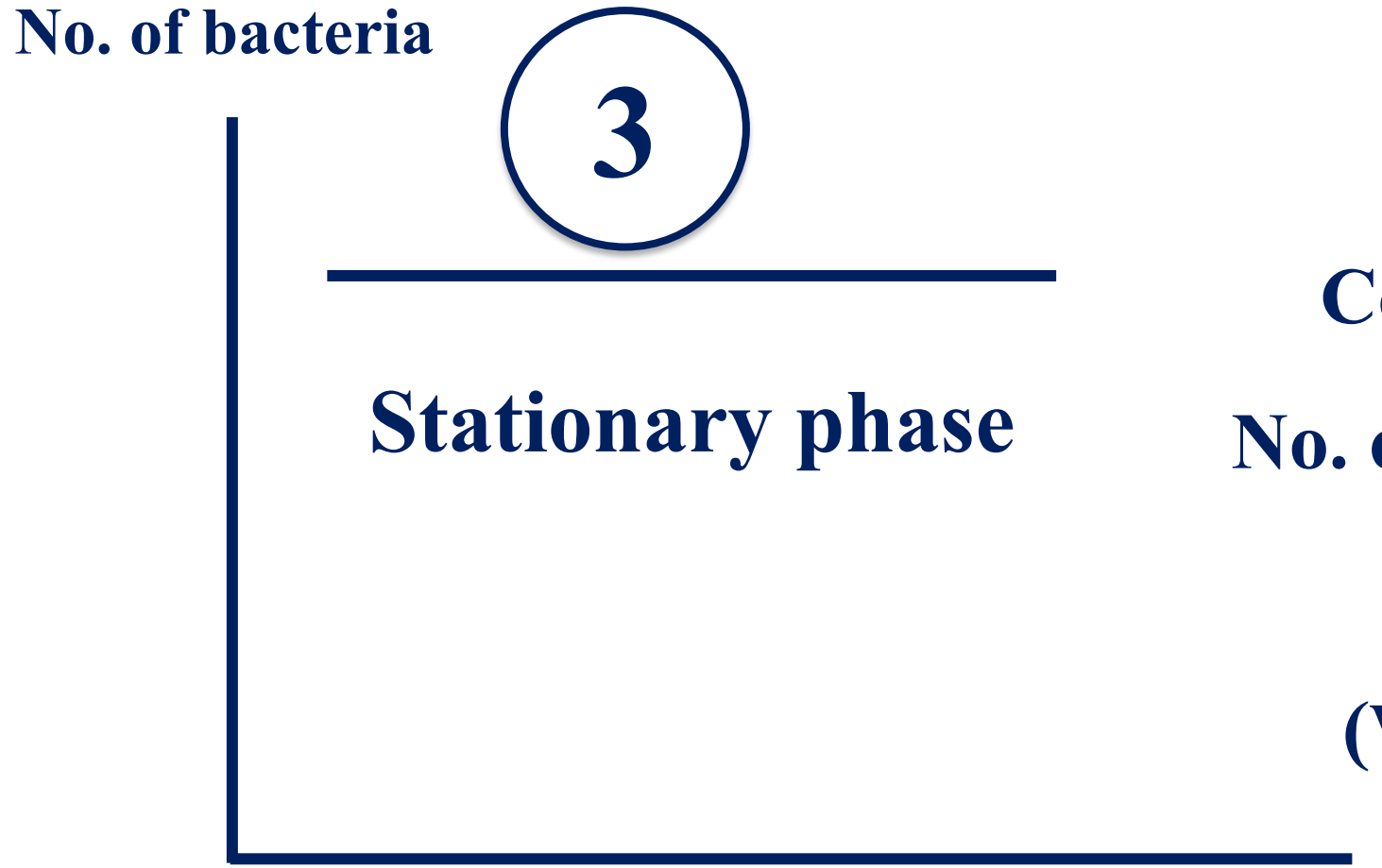
Lag phase


No. of bacteria
1
Lag phase
No. constant
&
Size
Since the bacteria still adapt and synthesize the needed enzymes and proteins.
Time/hrs

Log phase


No. of bacteria
Start division
2
Log phase
Number
Protein
Enzymes
This is the golden phase for bacteria, also it is the phase at which anti-biotics start their work by inhibiting bacterial growth.
Size
The size is reduced due to division
Time/hrs

Stationary phase



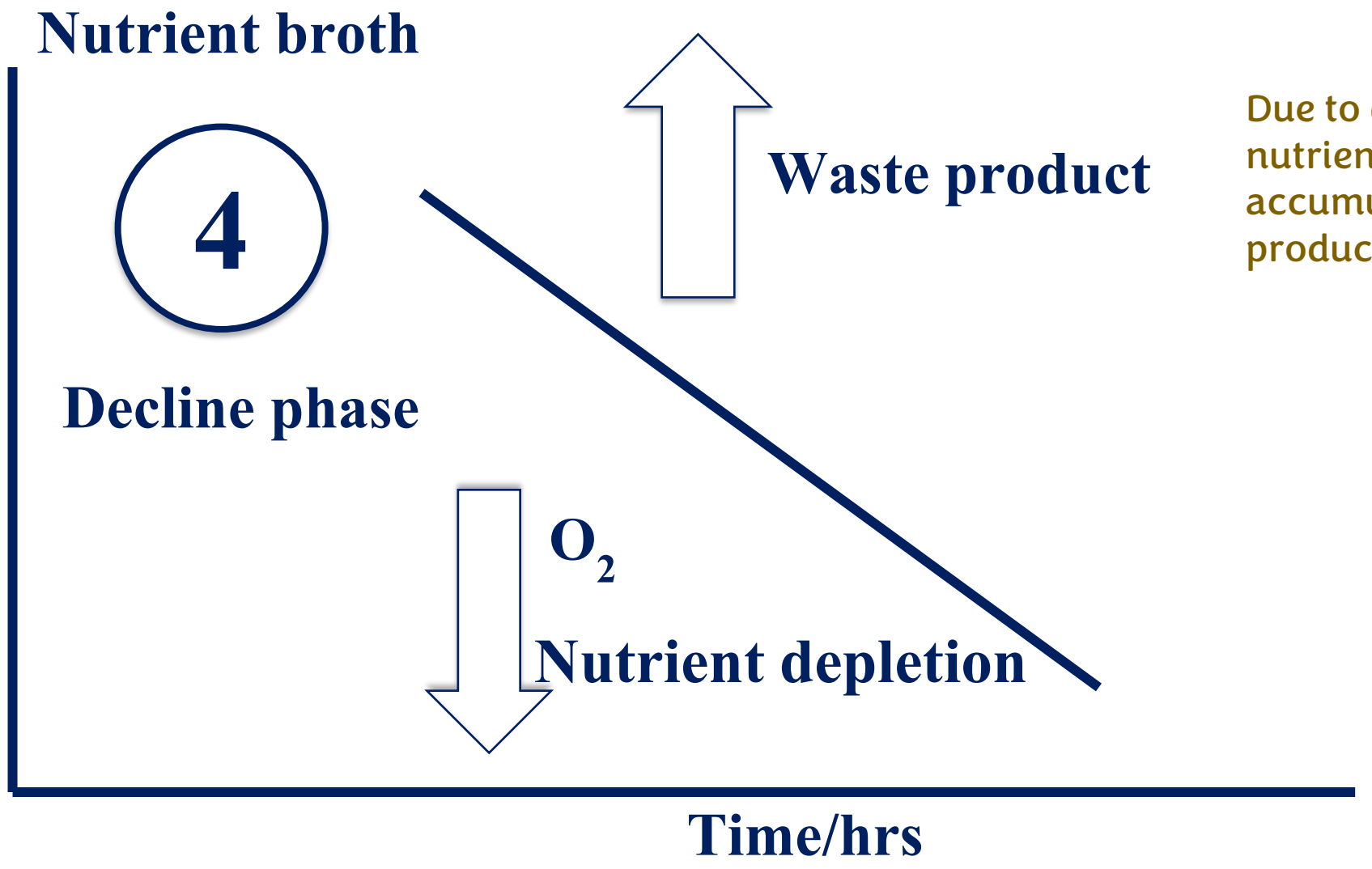
At this stage nutrients start to deplete, and waste products start to accumulate.

Constant number

No. of division = No. of death

(Waste product)

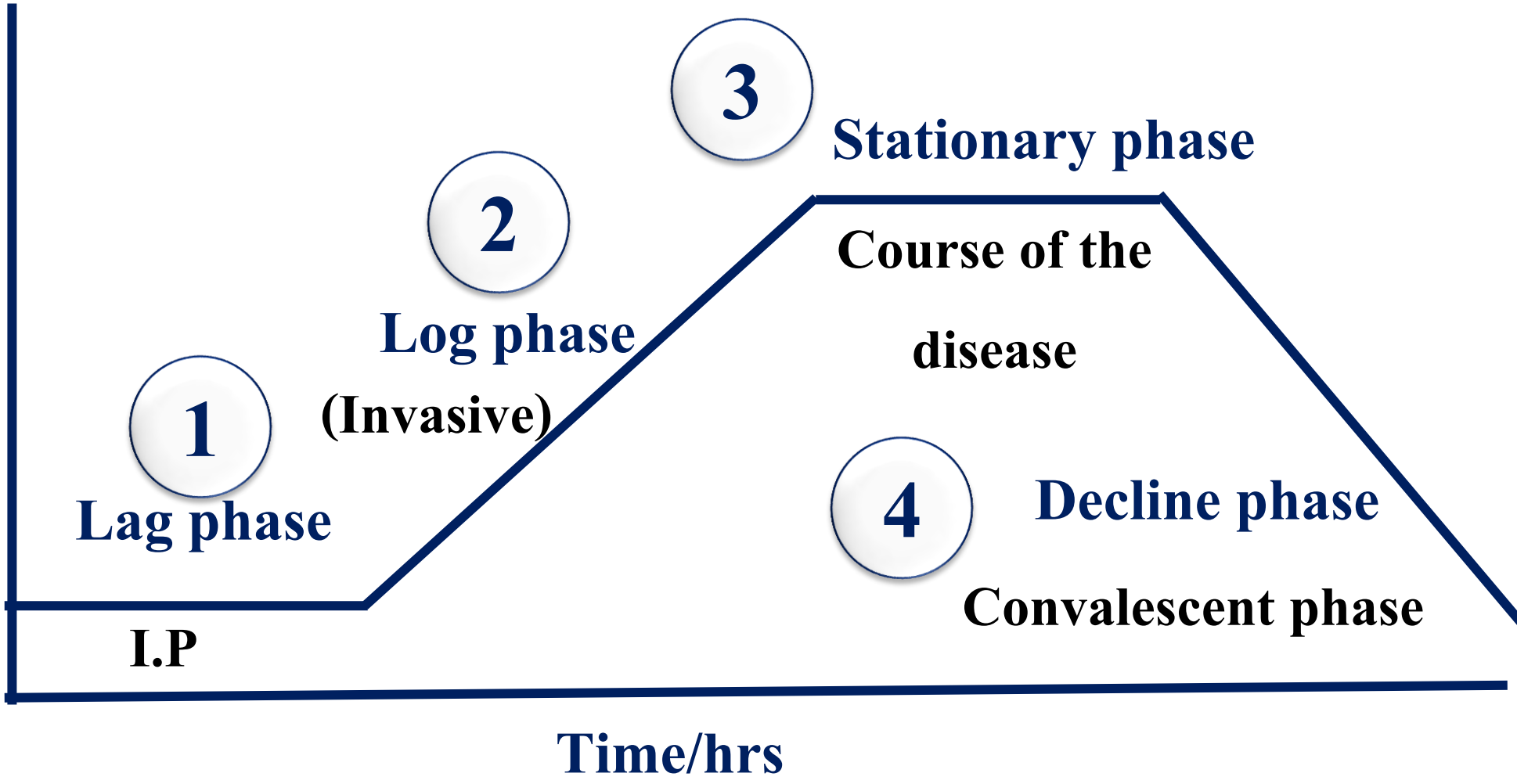
Decline phase



Due to depletion of nutrients and O_2, and accumulation of waste products, bacteria die.

Bacterial growth curve

The analogues for these phases in human body are:
1. incubation period
2. Invasive period (where symptoms start to appear)
3.Course of the disease
4.Convalscent phase (curing)



Bacterial growth requirements

Growth Requirements

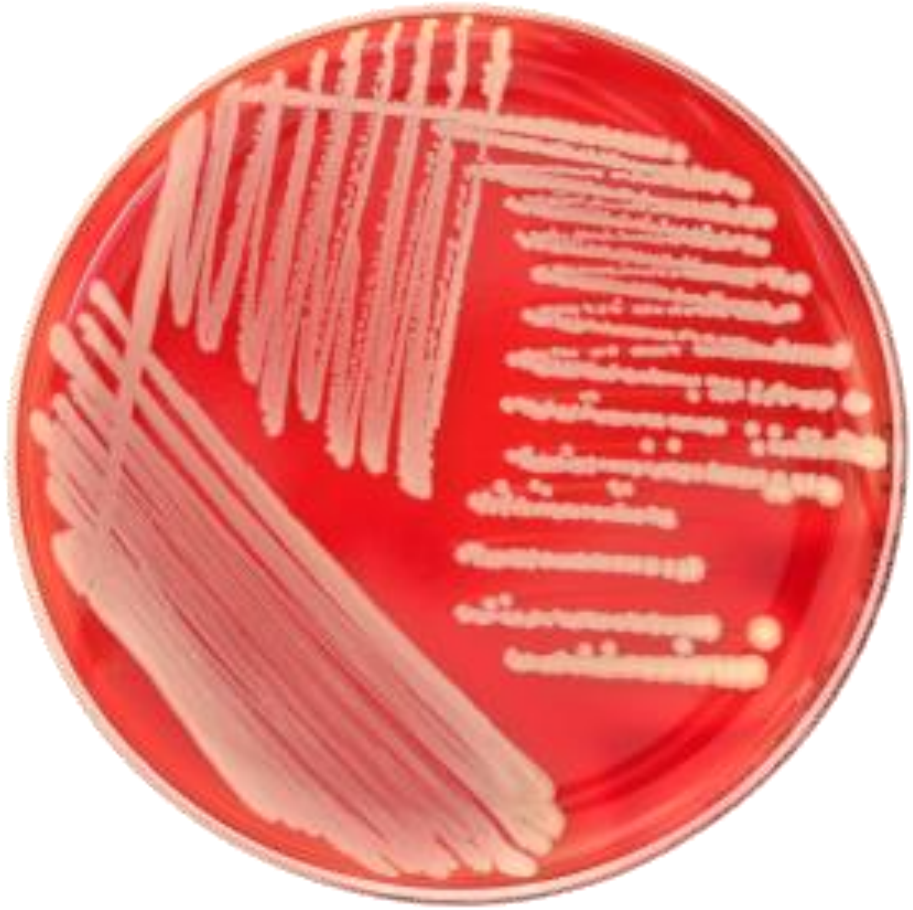
A) Nutrition

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

Carry out anabolism

CO_2

Carbon

Ammonium

Nitrogen

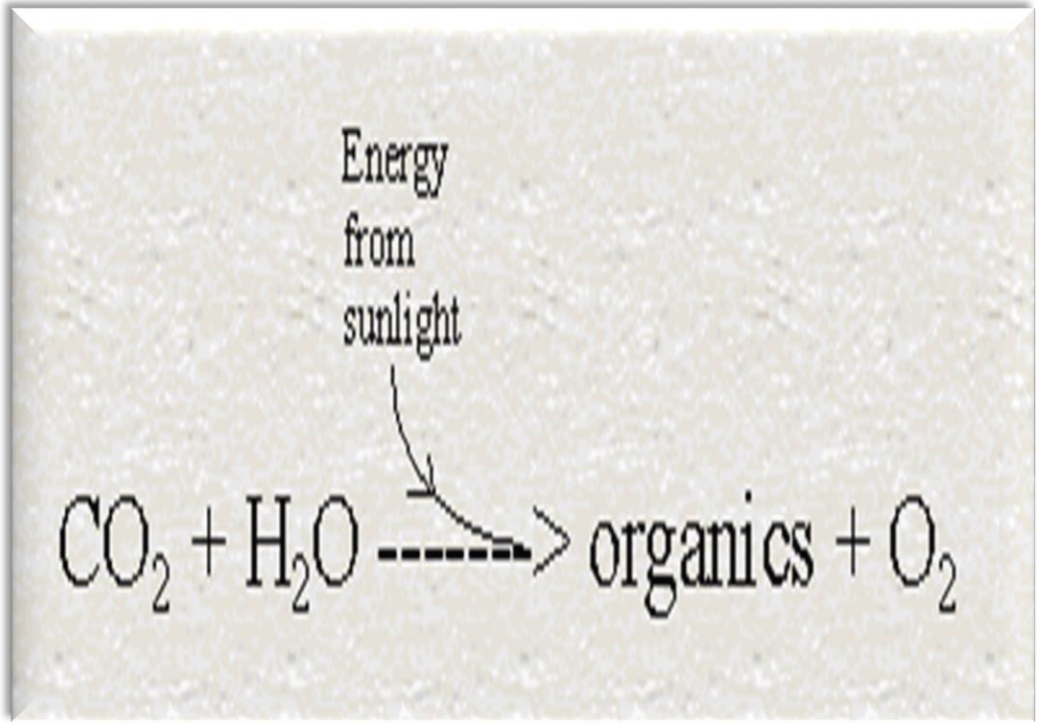
Complex organic materials

(Saprophytic)

Autotrophic

No medical importance

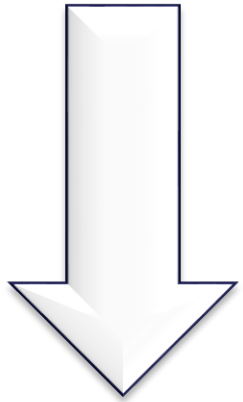
Since they are independent of other organisms in means of nutrition, they are not invasive, thus having no medical significance.



Heterotrophic

Parasitic

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



Living host

Since they are dependent on other organisms in means of nutrition, they are invasive, thus having a medical significance.

Medical important

Growth Requirements

Gaseous requirements

O_2 requirement, bacteria are classified into 5 groups

O2 requirement

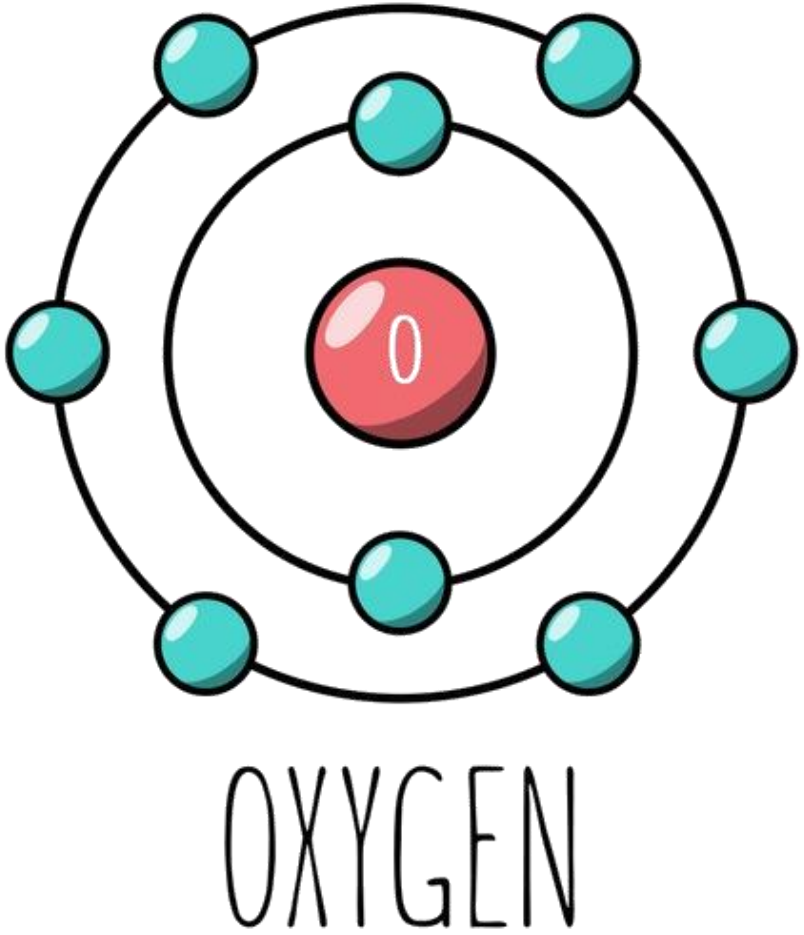
1) Obligate aerobes

2) Obligate anaerobes

3) Facultative anaerobes

4) Micro-aerophilic

5) Aero-tolerant



Respiration

Glucose catabolism

Energy production

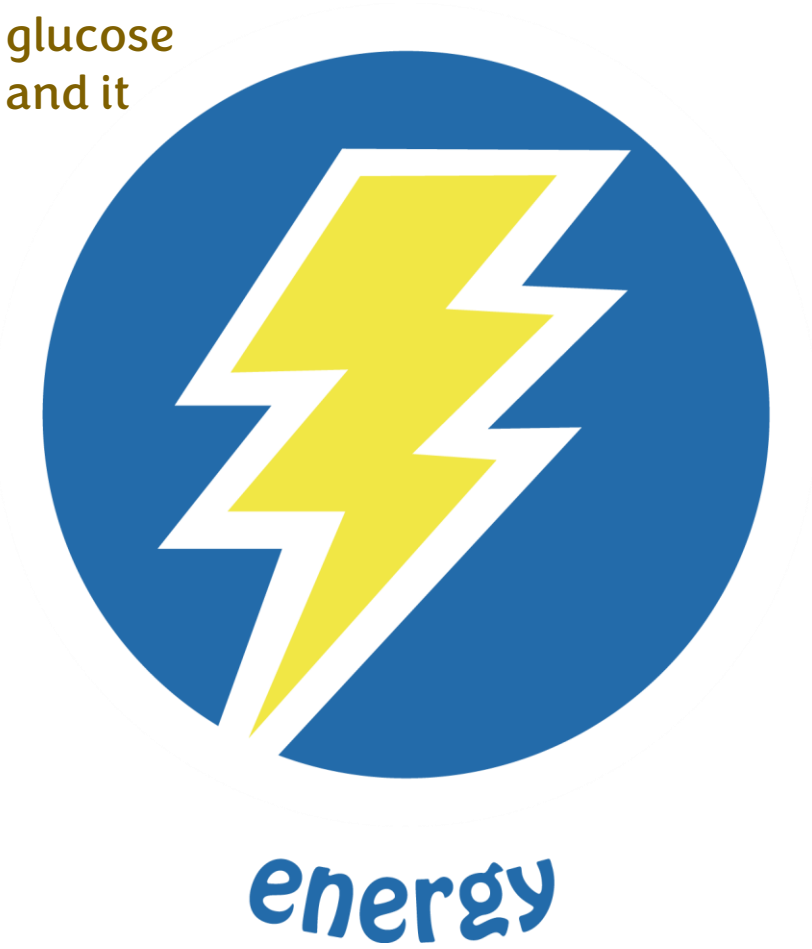
Aerobic respiration

(O_2)

Anaerobic respiration

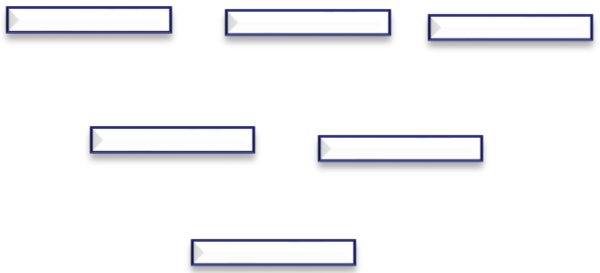
(No O_2)

Bacterial respiration is the catabolism of, mainly, glucose for energy production and it could be in absence or presence of O_2



1- Obligate aerobes (Aerobic respiration)

Presence of O_2



Growth

Absence of O_2

These bacteria cannot grow in the absence of O_2, since they cannot catabolize glucose without using O_2 as the final acceptor for electrons.

No growth

e.g. Pseudomonas aeruginosa

1- Obligate aerobes (Aerobic respiration)

Aerobic respiration

⇩

For production Energy (ATP)

⇩

Glucose catabolism (glycolysis)

1- Obligate aerobes (Aerobic respiration)

Glucose +ADP → 2 pyruvate + 2 ATP

$2NAD^+$ $2NADH$

2 pyruvate $\xrightarrow[\text{Cycle}]{\text{Kreb's}}$ $2FADH_2$ +<u>8NADH</u> + 2 ATP

Oxidative phosphorylation

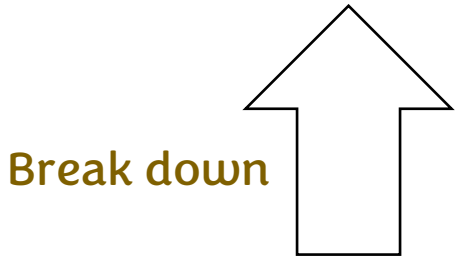
O_2 → Carry $2H^+$ → H_2O + 34 ATP

38 ATP

1- Obligate aerobes

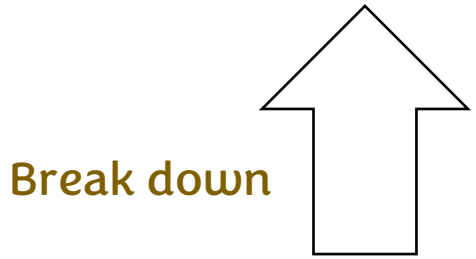
Highly toxic molecules

Superoxide (O_2)



Superoxide dismutase

(H_2O_2)



Catalase

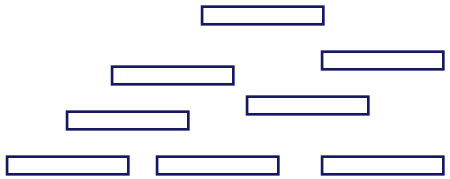
2- Obligate anaerobes

Presence of O2

These bacteria cannot survive in the presence of O_2, as they have not adapted to use it as an electron acceptor.

No growth

Absence of O2



Growth

Instead, these bacteria use other inorganic molecules as final electron acceptors, such as sulfate.

Bacteroides fragilis

2- Obligate anaerobes(Anaerobic respiration)

Glucose +ADP → 2 pyruvate + 2 ATP

$2NAD^+$ → 2NADH

2 pyruvate —Kreb's Cycle→ $2FADH_2$ +<u>8NADH</u> + 2 ATP

4 ATP

O_2 → Other pathway

Lack Superoxide dismutase &Catalase

So even if these bacteria used O_2 as an electron acceptor, toxic ROS would accumulate and cause their death

2- Obligate anaerobes (Anaerobic respiration)

The organism used inorganic molecules

- **Nitrate**
- **Sulfate**
- **Co2**

Carry H^+

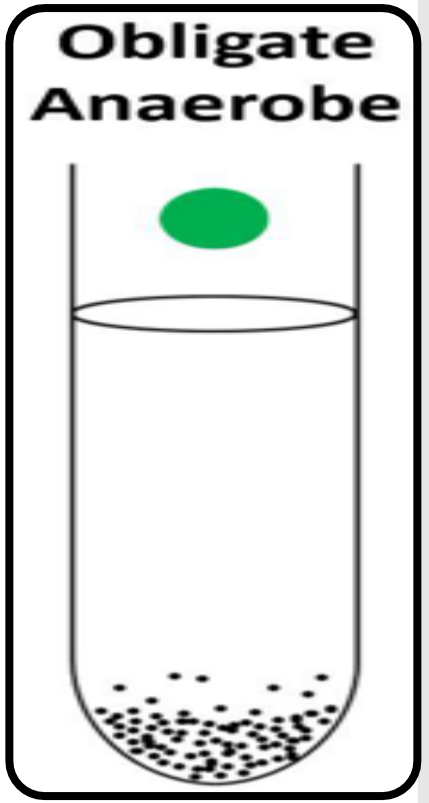
From the ETC.

From Kreb's cycle and glycolysis

13 ATP + 4 ATP

17 ATP

For more information regarding anaerobic respiration and alternative terminal electron acceptors in ETC refer to this.



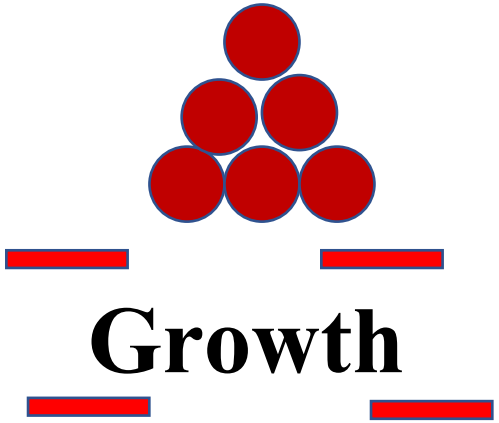
Lack

Superoxide dismutase

Catalase

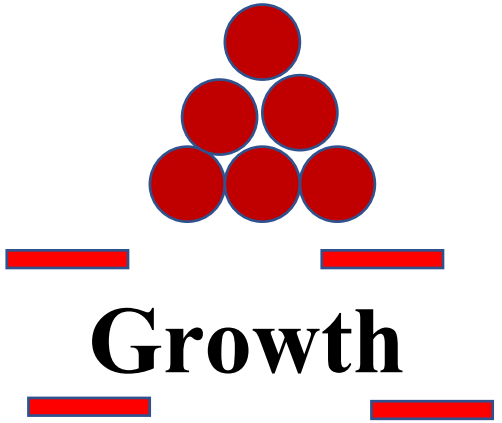
3- Facultative anaerobes

Presence of O_2



Growth

Absence of O_2



Growth

Rate of growth

Most bacteria

Originally, they live in the presence of O_2 exhibiting regular respiration where O_2 is the final electron acceptor , but they have adapted to live (less efficiently) in low O_2 levels or in its absence by using fermentation as a catabolic pathway.

3- Facultative anaerobes

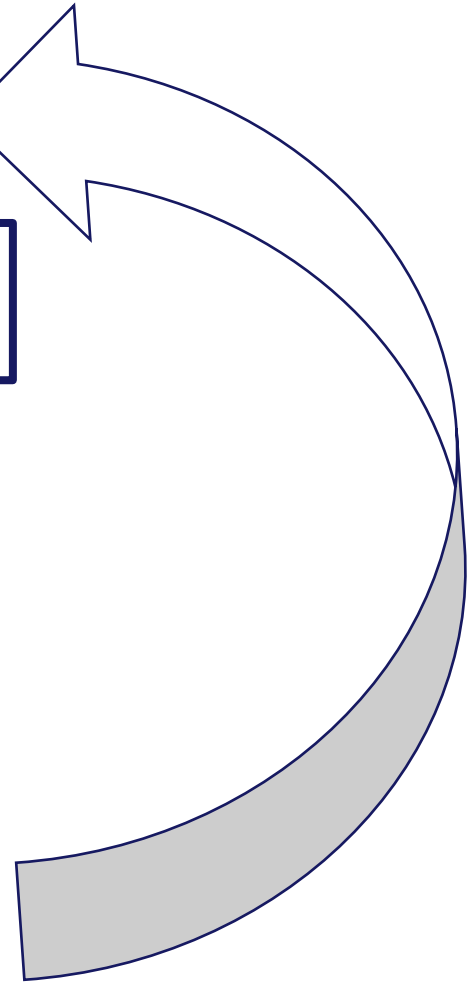
Anaerobes **Fermentation**

Glucose → **glycolysis** → **2 Pyruvate +** **2ATP**

No Kreb's cycle & other pathway

(Absence of carriers)

Acid & Alcohol



4- Micro-aerophilic

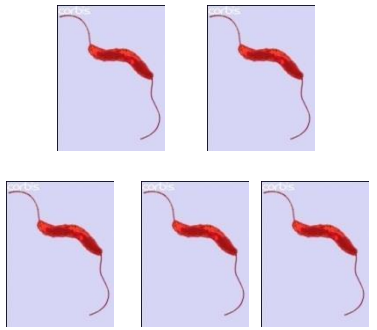
Presence of O_2

superoxide & H_2O_2

No growth

These bacteria can't live in normal levels of O_2 due to their decreased enzymatic activity of superoxide dismutase and catalase.

Low O_2



2-10% O_2

Growth

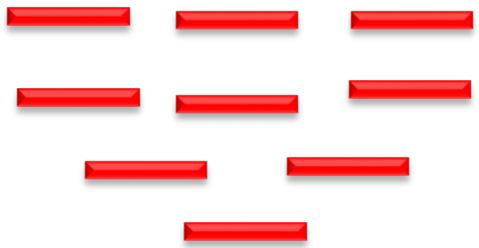
Campylobacter

Helicobacter

Low superoxide dismutase & catalase

5- Aero-tolerant anaerobes

Low O2



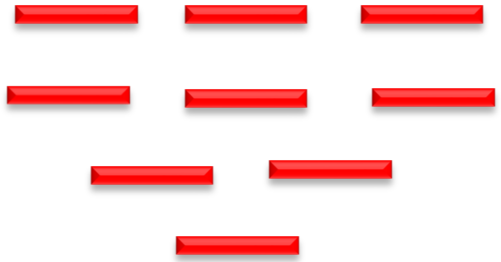
Superoxide dismutase

.

O_2 → H_2O_2

Tolerate

Absence of O2



Growth

Originally, they live in the absence of O_2 exhibiting respiration by using other inorganic molecules as final electrons acceptor, but they have adapted (tolerate) to live at low O_2 using it as the final acceptor of electrons for minutes before accumulation of ROS due to their decreased enzymatic activity.

Cl.perfringens

Growth Requirement: CO2 requirements

CO_2 (0.03%)

Present in air

is sufficient

For most of bacteria

CO_2 (5-10%)

(Capnophilic)

Neisseria

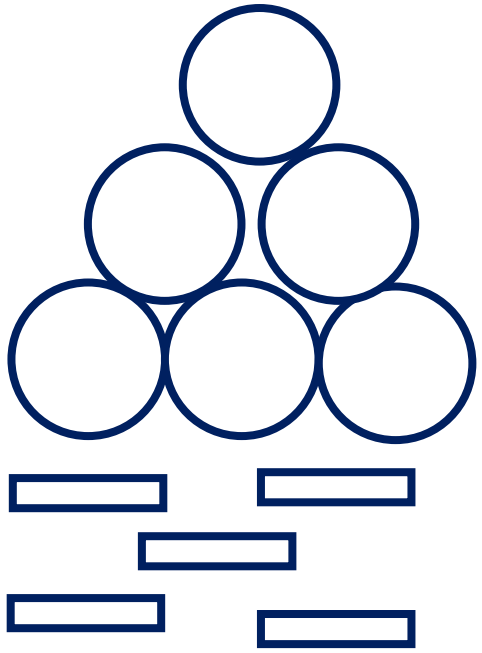
Brucella

Growth Requirement: Hydrogen ion (pH)

pH (7.2 – 7.4)

(Most bacteria)

And they are called Neutrophiles



Hydrogen ion (pH)

Alkaline (pH 9)

Vibrio cholerae

Alkaliphiles such as this prefer high pH levels.

Acidic (pH 4)

Lactobacilli

Acidophils such as this prefer low pH levels.

Growth Requirement: Temperature

Mesophilic

(20 – 45)

(Most bacteria)

psychrophilic

(0 – 15)

Thermophilic

(55– 65)

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

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	70	**pH (7.2 – 7.4)** **(Most bacteria)** And they are called Neutrophils neutrophils	**pH (7.2 – 7.4)** **(Most bacteria)** And they are called Neutrophiles neutrophiles
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
(numbered in order as cited in the text)

1. Science Direct
2. Share Biology

اللهم احفظ فلسطين وأهلها، والسودان وأهلها، ولبنان وأهلها وسوريا وأهلها
اللهم أحفظ أردننا الحبيب، وأهل أردننا وقيادة أردننا من كل شرٍ وفتنة ويسّر أمرنا لنصرة أهلنا في كل البلاد.