Bacterial Growth & Physiology

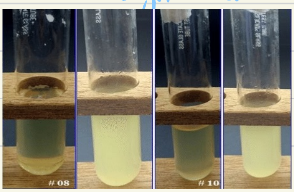
→ increase in the size & number of organisms

* Detection of bacterial growth *

a) on liquid meduim ; Broth

→ increased turbidity (cloudiness)

bacterial cloudiness is due to bacterial cells dispersing through medium



b) Detection on Solid media

→ apperance of visible colonies

→ macroscopic !!

↓

a single bacterium divides on a solid medium

→ after 20-30 divisions through binary fission a visible colony apears

↓

which is the macroscopic result of many bacterial cells orginating from a single bacterium

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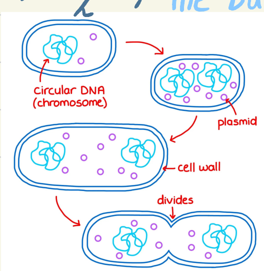
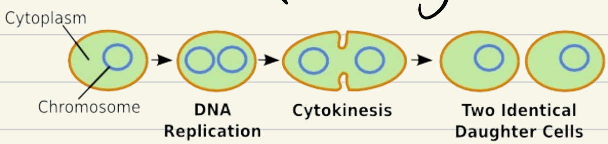
quantitative Prespective on bacterial growth

→ after 20-30 rounds of binary fission, bacterial population can reach approx. 1 million cells

→ 2^{20} ; the base 2 represent division of one cell into two cells & 20 signifies number of divisions



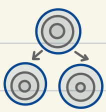
Generation time (or doubling time) → The time it takes for a bacterial cell to undergo division & double its population

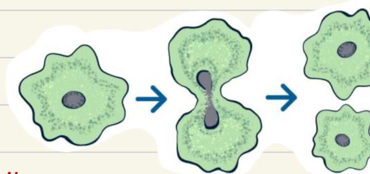


V. cholerae
↓
fast generation time
↓
13 mins
↓
meaning it can double its population in this short period

M. Tuberculosis
↓
very slow; 24 hours
↓
slow rate of division

Bacterial reproduction / binary fission

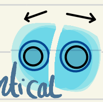




→ a single bacterial cell divides to produce two genetically identical daughter cells

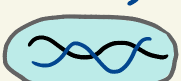
⇉ Steps ⇇

⑤ cell sepration



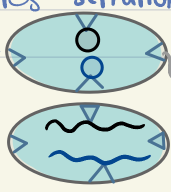
Producing two identical daughter cells

① **Elongation** of the bacterial DNA. This process occurs in a single direction, ensuring daughter cells will be the same size



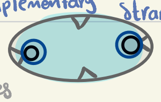
↳ made linear for simplification

② **Sepration of the 2 strands** each ssD becomes attatched to mesosome, the enzyme facilitates sepration of Ds into ss

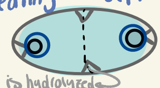


mesosom: a structure within bacterial cell that provides a site for enzymatic activity

③ **Seprate ssDNA & become ds DNA** each ssDNA serves as a template to synthesize complementary strand



④ **Formation of division Septum** The bacterial mb & Peptidoglycan cell wall begin to grow inward creating a septum



septal Po is hydrolyzed

→ it's artificial !! (specifically formulated to mimic natural conditions)

{Bacterial culture media} → For bacterial growth in vitro (Bacterial growth outside biological environment), a suitable culture medium must provide essential nutrients

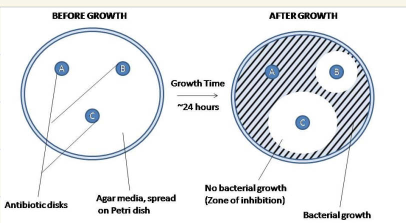
Purpose? to determine if a patient has a bacterial infection

1. Study properties of bacteria → charateristics help in identifying & differentiating bacterial species

2. Isolation & diagnosis (causative agent)
   - allowing specific colonies to grow
   - identifying specific type of bacteria responsible for infection

3. Prepare vaccine & other products → for ex. S. Pyogenes can be used in anticoagulants

4. Selecting proper antibiotic → identifying most effective antibiotic for treating bacterial infection



Classification of media

- liquid → in a tube & we call it broth
- Solid / semi-Solid → Petri dish

Types of media

1. Simple media

→ Serve as the basic nutrient sources required by most bacteria for growth, they provide minimal ingredients necessary for bacteria to thrive in a lab setting

(A) Peptone water

consist of protien Source & 0.5% Nacl
↓
enhance bacterial growth specially when supplemented with sugar

(B) Nutrient broth

↓
contains meat extract providing essential nutrients that supports bacterial growth

(C) Nutrient agar plate

↓
Nutrient broth + 2% agar agar
↓
derived from seaweeds acts as a gelling agent
↓
Suitable for : staph. aureus

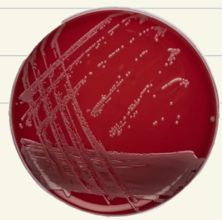
2. Enriched media

: fastidous bacteria → have complex nutritional requirments & need Blood & Serum to grow

(A) Blood Agar

↓
nutrient-rich by ading sheep blood to nutrient agar. The agar is heated to 45°C to create a semisolid medium before adding (sheep blood)

→ streptococci
→ strept. Pyogenes → Exhibit hemolytic activity!!



Hemolysis on a blood agar

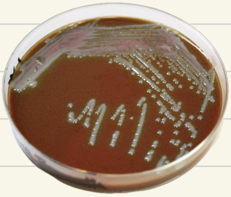
Ⓐ Complete (Beta) hemolysis :
1. Staphylococcus aureus
2. Streptococcus Pyogens

Ⓑ Partial (alpha) hemolysis
1. Streptococcus Viridans
2. Pneumo cocci

Ⓒ No hemolysis (gamma)
- Enterococci

https://questions.widenex.com/3ee7f4b3-b4b7-4e34-8151-054397a60801 ← Quiz yourself !!

Enriched media Ⓑ chocolate agar



Prepared by heating nutrient agar to 100°c & then adding blood

The high tempreture causes hemoglobin in RBCs to breakdown into hematin → which gives medium its charaterstic brown color

- Haemophilus
- Neisseria

3. Selective media → Specialized types of growth media used to cultivate specific microorganisms while suppersing growth of others.

This is achived by adding certain chemicals, dyes, antibiotics or other compounds that inhibit the growth of unwanted microorganisms, thus allowing only the desired species or group of organisms to thrive

Löwenstein Jensen medium
- isolating Mycobacterium Tuberculosis
- Selective agent here is Malachite green
  which inhibits non-myobacterial organisms

Blood tellurite agar
- Selective for C. diptheriae
  inhibiting other bacteria

4. Differntial media → combine Selective properties with indicators to allow specific organisms to grow while revealing differences between bacteria by a visible indicator change

Macconkey's Agar: differntiate bacteria that can ferment lactose from those that cannot

components →
- Bile Salts : Selective for Enterobacteria
- lactose : Test sugar
- peptone : Source of nitrogen for bacterial growth
- Natural red : PH indicator

* Bacteria that ferment lactose produce acid, turning medium pink

* Non-lactose fermenters produce no acid & appear pale or yellow

Mannitol Salt Agar :

↳ differntiates staphoccus based on their ability to ferment mannitol

staph.spp

↓

components :

* High Salt conc. (7.5% Nacl)
* Mannitol : fermentable sugar
* Phenol red: A PH indicator that turns yellow in acidic conditions

Thiosulfate - citrate - Bile - sucrose (TCBS)

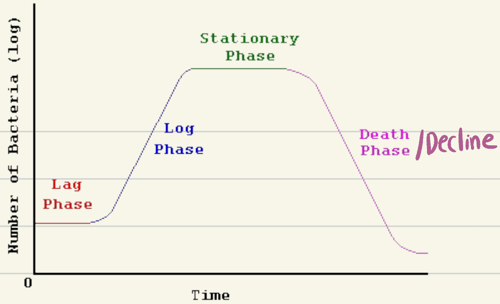
↳ differntiating vibro species

* Thiosulfate * Bile * sucrose * citrate * Bromothymol blue → PH indicator

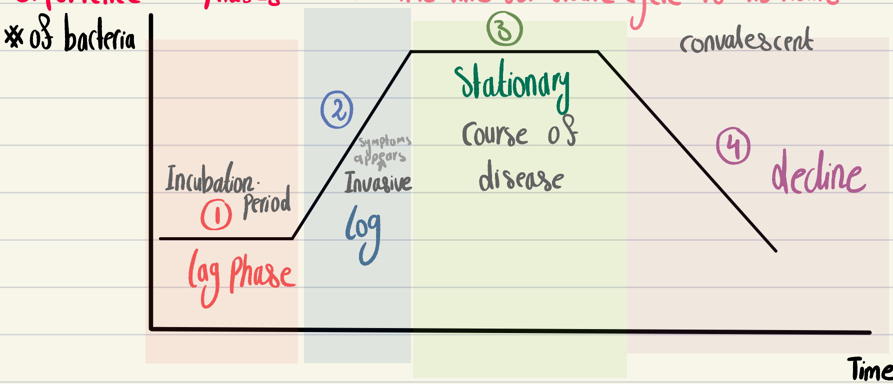
* vibro cholera : ferment sucrose, producing acidic by-products → turns yellow
* vibro Paraheamolyticus : medium remains green

﴿وَتَوَكَّلْ عَلَى اللَّهِ وَكَفَى بِاللَّهِ وَكِيلا﴾

Bacterial growth curve



If a small number of bacteria are inoculated into a liquid nutrient medium they experience 4 Phases → time line for entire cycle is 72 hours



← analogy between bacterial growth curve & Progression of disease in human body

① lag phase: bacteria adapt to new enviroment
focus on Synthesizing essential enzymes & protiens needed for growth
number of bacteria remain constant but cells increase in Size

② Log Phase: bacteria Start rapid cell division → exponential growth in Population
"golden phase": rate of bacterial growth is at its peak; antibiotics are most effective here
Due to cell division, individual cell size may decrease

③ Stationary phase: resources become limited & waste products accumul, rate of cell division slow down & becomes equal to rate of cell death → Stable Population size

④ decline Phase: nutrients are almost entirely depleted & waste Products accumulated to toxic levels
oxygen & other resources are exhausted → ↓↓ in ※ of cells

Bacterial growth requirments → A) Nutrition B) Gaseous C) Temp. & pH

A) Nutrition - Maintenance of bacterial growth

Autotrophic
Self - nutrition

→ utilize simple inorganic Substances like CO_2 & ammonium

utilize simple inorganic Substances [CO_2 ↓ carbon, ammonium ↓ Nitrogen] into complex organic material

[Saprophytic]

No medical importance → They don't rely on infecting a host for growth

Hetrotrophic
different nutrition
↓
require complex preformed organic substances like sugars & protiens
↓
Due to their dependency on a host, these bacteria can invade the host's tissues leading to infections & diseases
↓
medically significant

B) Gaseous requirments - O_2 requirments - 5 groups

1) obligate aerobes
2) obligate anaerobes
3) faculative anaerobes
4) Micro - aerobic
5) Aero - tolerant

Respiration

break down of glucose - catabolism - release of Energy

- Aeorbic ↓ requires O_2 yeilds a high amount of energy
- Anaerobic ↓ without oxygen

1. obligate aerobes

* depends on presence of oxygen for growth

* without O_2 → no growth or survival

ex. Psuedomonas aeruginosa
↓
aerobic respiration → production of ATP
→ glucose catabolism (glycolysis)

glycolysis → kreb's cycle → oxphos → 38 ATP
↓
produces highly toxic materials
↙ Superoxide O_2^- — superoxide mutase breaks it down
↘ H_2O_2 — catalase breaks it down

2. obligate anaerobes

→ O_2 is toxic to them!!!

* no growth in O_2 presence

* growth in absence of O_2
↓
Ex. Bacteroides fragilis

4ATP

* lack superoxidase dimutase & catalase

* organism use inorganic molecules
↙ Nitrite (NO_3^-) ↓ Sulfate ↘ CO_2

To carry e^- in ETC
↓
this process generates ATP but less efficient than aerobic
↓
13 ATP from ETC + 4 ATP from glycolysis

3. Faculative anaerobes → orginally lives in enviroments with oxygen. However, they are adaptable & can survive in low or no O_2

Presence of O_2

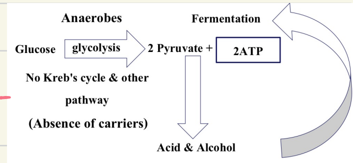
↓

↑ generate a high rate of ATP → ↑ increased growthrate

Absence of O_2

↓

Switch to anaerobic Pathways, such as fermentation to Produce energy



when O_2 is absent, faculative anaerobes rely on glycolysis to break down glucose into Pyruvate producing a net gain of 2 ATP molecules per glucose → no kreb's cycle or ETC

after glycolysis, Pyruvate is further metabolized in fermentation process leading to production of lactic acid & alcohol

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4. Micro-aerophilic

Ex. campylobacter & Helicobacter

Presence of O_2

↓

No growth !!!

Because they Produce toxic by-products

- Superoxide (O_2^-)
- H_2O_2

→ low levels of Superoxide dimutase & catalase

low O_2 conditions (2 - 10%)

↓

growth ✓✓

↓

low level of O_2

↓

grow without accumulating toxic levels of O_2^- & H_2O_2

5. Aeoro-tolerant anaerobes → Ex. clostridium perfringens

- low O_2 conditions
  - can survive in enviroments with low oxygen, They posses superoxide dimutase ✓✓
- absence of O_2
  - ↓ grow and carry out typical metabolic processes ✓
  - ↓ uses inorganic molecules as the final e^- acceptor

Growth requirment : CO_2 requirments

- Standard CO_2 requirment (0.03%)
  - ↓ Suffient for growth for most bacteria
- CO_2 (5-10%)
  - requires higher level of CO_2
  - ↓ Neisseria & brucella

Growth requirment : PH

Neutral (7.2-7.4)	alkaline (PH = 9)	acidic (PH=4)
* Most bacteria * called Neutrophils	↓ Ex. vibro cholerae	Ex. lactobacilli Acidophiles

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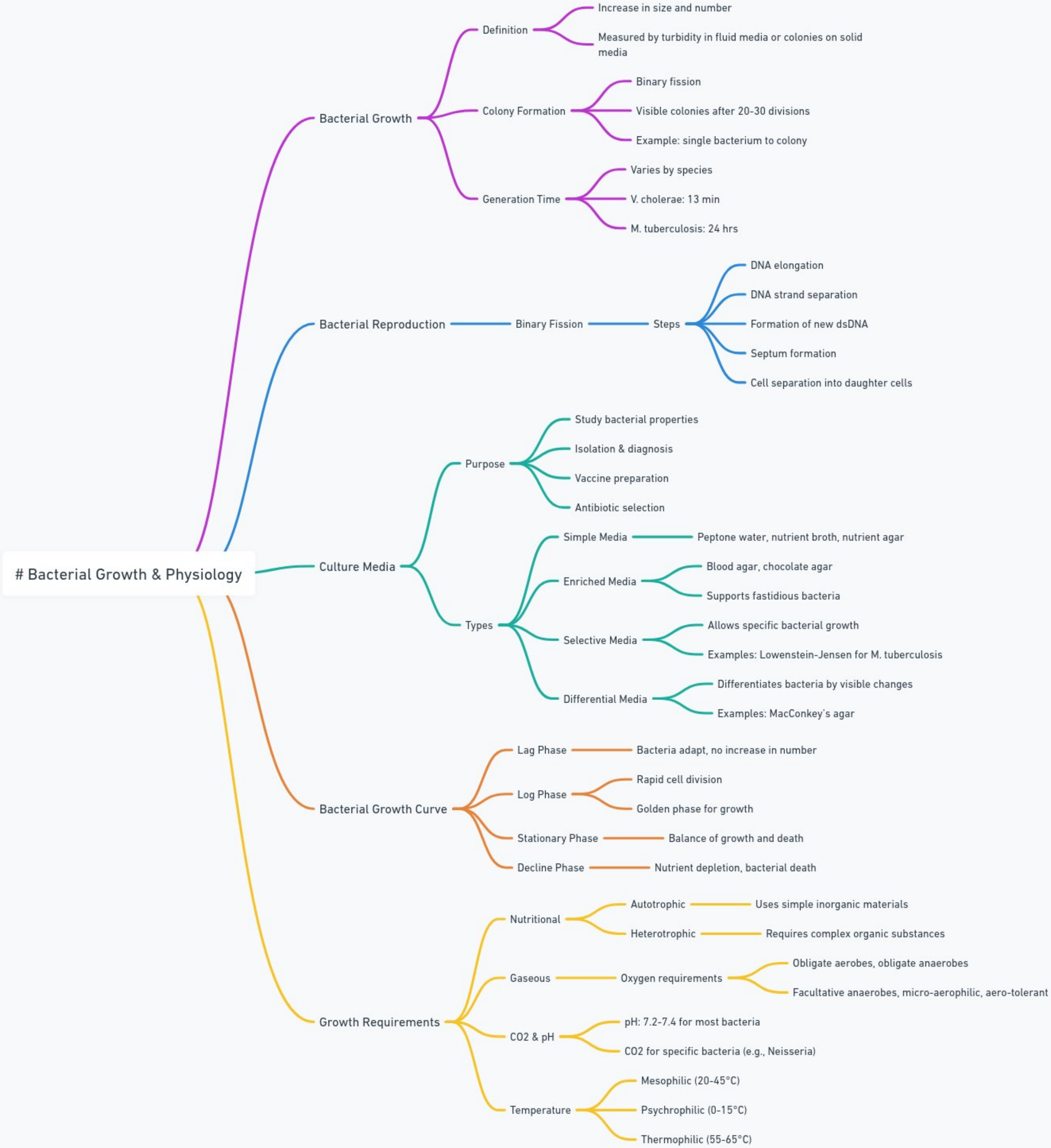
Growth requirment : temprature

Mesophilic	Psychrophilic	thermophilic
(20 - 45) Most bacteria	(0 - 15)	(55 - 65)



Bacterial Growth & Physiology
Bacterial Growth
Definition
Increase in size and number
Measured by turbidity in fluid media or colonies on solid media
Colony Formation
Binary fission
Visible colonies after 20-30 divisions
Example: single bacterium to colony
Generation Time
Varies by species
V. cholerae: 13 min
M. tuberculosis: 24 hrs
Bacterial Reproduction
Binary Fission
Steps
DNA elongation
DNA strand separation
Formation of new dsDNA
Septum formation
Cell separation into daughter cells
Culture Media
Purpose
Study bacterial properties
Isolation & diagnosis
Vaccine preparation
Antibiotic selection
Types
Simple Media
Peptone water, nutrient broth, nutrient agar
Enriched Media
Blood agar, chocolate agar
Supports fastidious bacteria
Selective Media
Allows specific bacterial growth
Examples: Lowenstein-Jensen for M. tuberculosis
Differential Media
Differentiates bacteria by visible changes
Examples: MacConkey's agar
Bacterial Growth Curve
Lag Phase
Bacteria adapt, no increase in number
Log Phase
Rapid cell division
Golden phase for growth
Stationary Phase
Balance of growth and death
Decline Phase
Nutrient depletion, bacterial death
Growth Requirements
Nutritional
Autotrophic
Uses simple inorganic materials
Heterotrophic
Requires complex organic substances
Gaseous
Oxygen requirements
Obligate aerobes, obligate anaerobes
Facultative anaerobes, micro-aerophilic, aero-tolerant
CO2 & pH
pH: 7.2-7.4 for most bacteria
CO2 for specific bacteria (e.g., Neisseria)
Temperature
Mesophilic (20-45°C)
Psychrophilic (0-15°C)
Thermophilic (55-65°C)

Bacteria	Characteristics/Notes	Media or Conditions
V. cholerae	Rapid division time (13 minutes)	Thiosulfate-Citrate-Bile-Sucrose (TCBS) Agar
M. tuberculosis	Very slow division time (24 hours)	Lowenstein Jensen medium
Streptococcus pyogenes	Beta-hemolytic	Blood agar
Streptococcus viridans	Alpha-hemolytic	Blood agar
Staphylococcus aureus	Beta-hemolytic, can ferment mannitol	Blood agar, Mannitol salt agar
Staphylococcus epidermidis	Cannot ferment mannitol	Mannitol salt agar
Enterococci	Gamma-hemolytic	Blood agar
Neisseria	Fastidious, requires enriched media, capnophilic (5-10% CO_2)	Chocolate agar
Haemophilus	Fastidious, requires enriched media	Chocolate agar
Corynebacterium diphtheriae	Grows on selective medium	Blood tellurite agar
Pseudomonas aeruginosa	Obligate aerobe	General media, requires oxygen
Bacteroides fragilis	Obligate anaerobe	Grows in absence of oxygen
Campylobacter	Microaerophilic (low oxygen requirement)	Requires reduced oxygen levels
Helicobacter	Microaerophilic (low oxygen requirement)	Requires reduced oxygen levels
Clostridium perfringens	Aero-tolerant anaerobe	Can tolerate low oxygen
Brucella	Capnophilic (requires increased CO_2)	Requires 5-10% CO_2
Lactobacilli	Acidophilic, prefers acidic environments	Prefers low pH (around 4)
Vibrio cholerae	Alkaliphilic, prefers basic environments	Prefers high pH (around 9)