Microbiology

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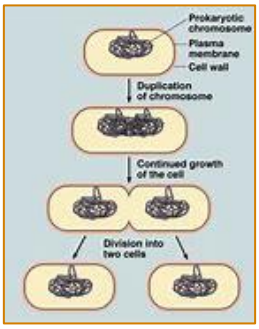
ARKAN
ACADEMY

Bacterial Growth& physiology

- **Bacterial Growth**
  - Increase in *size* and *number* of organism
  - Indicated by:
    1. Turbidity of the fluid media (broth)
    2. Colonies on solid media (Macroscopic product)
       - ✓ *Colony*: is a group of bacteria that originates from a single bacterium *cultured* on solid media. after 20-30 divisions (through binary fission)
       - ✓ The number of colonies will reach approximately 1 million (2^20) after 20-30 divisions of a single bacterium.
       - ✓ Generation time (doubling time) varies between different bacteria:
         - Rapid: 13min (*V.cholerae*)
         - Slow: 24 hrs (*M.tuberculosis*)

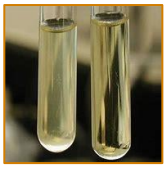
- Bacterial reproduction by **Binary fission**
  1. *Elongation* (in one side of DNA)
  2. *Separation* of 2 strands (ssDNA attached to mesosomes by their enzyme separate each strand)
  3. *Separate* ssDNA & become dsDNA
  4. *Formation* of division septum
  5. *Cell separation* (2 daughter cells)



- Bacterial culture **media**: [Artificial]
  - Bacteria grow (In vitro) → Need nutrients for growth
  - *Purpose*:
    - ✓ *Study* Properties
    - ✓ Isolation & *diagnosis* (Causative agent)
    - ✓ Prepare *vaccine* & Other product
    - ✓ For selection proper *antibiotics*

- **Classification** of media:
  - Liquid (*broth*)
  - Solid (*agar*)

- Types of media
  - A. Simple media
    - Basic requirement for growth of most bacteria
      1. *Peptone water*
         - ✓ Peptone + 0.5% NaCl
         - ✓ Purpose: Enhancement
         - ✓ Sugar media
      2. *Nutrient broth*
         - ✓ Meat extract
         - ✓ Enhancement
      3. *Nutrient agar plate*
         - ✓ Nutrient broth + 2% agar agar (Seaweed)
         - ✓ *Staph. aureus*

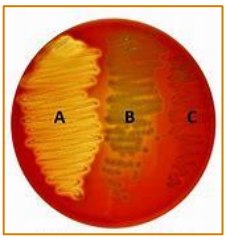


B. Enriched media

- Fastidious bacteria :need blood or serum for growth

1. *Blood agar*

- ✓ Nutrient agar heated at 45°C (semisolid) + sheep blood
- ✓ *Streptococci/ Strept. Pyogenes*
- ✓ Haemolysis on blood agar:
  - Complete (***beta***) haemolysis: [clear]
    - *Staphylococcus aureus*
    - *Streptococcus pyogenes*
  - Partial (***alpha***) haemolysis: [green]
    - *Streptococcus viridans*
    - *Pneumococci.*
  - No (***gamma***) haemolysis: [no change]
    - *Enterococci.*



2. *Chocolate agar*

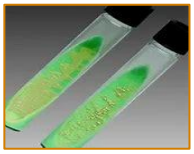
- ✓ Nutrient agar heated at 100°C, add blood
- ✓ Hb → Heat→ Haematin (Chocolate)
- ✓ *Haemophilus Neisseria*

C. Selective media

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

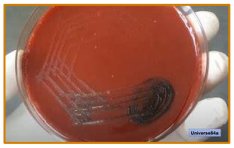
1. *Lowenstein Jensen medium*

- ✓ Selective material Malachite green
- ✓ *Mycobacterium tuberculosis*



2. *Blood telluriteagar*

- ✓ Selective material Potassium tellurite
- ✓ *C.diphtheriae*



D. Differential media

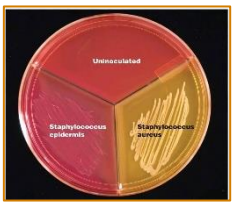
- Selective (Allow a certain organism to grow) + Indicator (Indicator to differentiate (change in visibly))

1. *MacConkey's agar*

- ✓ Bile (selective to Enterobacteria) +Lactose(test sugar)+ Peptone+ Neutral red (pH indicator)
- ✓ If *can* fermentation lactose the color appear : ***pink***
- ✓ If *cannot* fermentation lactose the color appear :***pale***

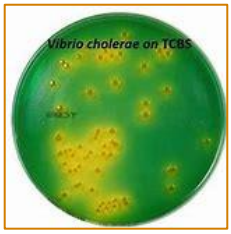
2. *Mannitol salt agar*

- ✓ (high salt 7.5% NaCl) +Phenol red (pH indicator)
- ✓ Staphylococcus aureus can ferment the mannitol sugar color appear: ***yellow***



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

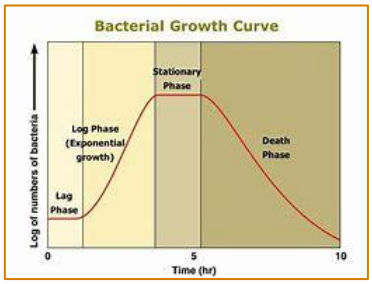
- ✓ Thiosulphate +Bile + Citrate [selective] + Sucrose [test sugar]+ Bromothymolblue [indicator]
- ✓ *V.cholera* can ferment sugar from green to yellow



- **Bacterial growth curve**
  - ***Definition:*** If a small number of bacteria are inoculated into a liquid nutrient medium, within 72 hours they will go through the following phases:

1. *Lag phase*:
   - ✓ There is little to *no increase* in the number of cells. However, metabolic activity is *high*, and cell size increases as the bacteria prepare for growth.
2. *Log Phase (Exponential Phase):*
   - ✓ The number of bacteria, along with protein and enzyme production, *increases* rapidly. However, cell size *decreases* due to the high rate of cell division.
3. *Stationary phase*:
   - ✓ There is an *equilibrium* between cell division and death (constant number)
4. *Decline phase (Death phase):*
   - ✓ The number of deaths *exceeds* the number of new cells formed. (nutrients and O2 start to deplete & toxic materials start to be increase)

- Bacteria growth curve in **human body**:
  1. Lag phase : *Incubation period*
  2. Log phase: *Invasive* start symptoms and sign
  3. Stationary phase: *Course* of the disease
  4. Decline phase : Convalescent phase (*recovery*)



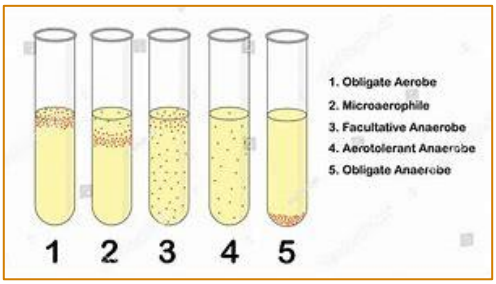
- **Bacterial growth requirements**

A) Nutrition

- to Maintenance of bacterial growth
- *Autotrophic*
  - ✓ [auto= self / Trophic=nutrition]
  - ✓ Utilize simple inorganic substance
  - ✓ Uses CO_2 as a carbon source and ammonium as a nitrogen source to produce complex organic materials. (*saprophytic*)
  - ✓ *No* medical importance
- *Heterotrophic*
  - ✓ [hetero= different/ Trophic= nutrition]
  - ✓ These bacteria require complex, preformed organic substances (e.g., sugars, proteins) from living cells and typically live as parasites
  - ✓ *Medical* important

B) Gaseous

- Respiration:
  - ✓ Glucose catabolism → Energy production
    - Aerobic respiration (O2)
    - Anaerobic respiration (No O2)
- O2 requirement, bacteria are classified into 5 groups:



1. Obligate aerobes (Aerobic respiration)
   - ✓ *Presence* of O2 to growth
   - ✓ Aerobic Respiration: The production of *energy* (ATP) through the catabolism of glucose, primarily via glycolysis, in the presence of *oxygen*
   - ✓ e.g. *Pseudomonas aeruginosa*

- ✓ *Pathway* :
  - Glucose +ADP → 2NAD+ 2NADH→ 2 pyruvate + 2 ATP
  - 2 pyruvate→ Kreb's cycle → 2FADH2 +8NADH + 2 ATP
  - [Oxidative phosphorylation] O2 → Carry 2H → H2O + 34 ATP
  - The result: 38 ATP + Highly toxic molecules [Superoxide and hydrogen peroxide]
- ✓ *Bacterial Enzymes* to Remove Toxins:
  - *Superoxide Dismutase*: Cleaves superoxide (O_2) into less harmful molecules.
  - *Catalase*: Cleaves hydrogen peroxide (H_2O_2) into water and oxygen

2. Obligate anaerobes (Anaerobic respiration)
   - ✓ Can growth in *absence* of O2
   - ✓ E.g. *Bacteroides fragilis*
   - ✓ Pathway:
     - Glucose +ADP → 2NAD+ 2NADH→ 2 pyruvate + 2 ATP
     - 2 pyruvate→ Kreb's cycle → 2FADH2 +8NADH + 2 ATP
     - No O2 → Other pathway
     - The organism used inorganic molecules : Nitrate/ sulfate /Co2 → Carry H^+ +13 ATP

       The result:13 ATP + 4 ATP =17 ATP
   - ✓ *Lack* Superoxide dismutase and Catalase so don't produce toxic material

3. Facultative anaerobes
   - ✓ These bacteria can grow in both the *presence* and *absence* of oxygen. However, their growth rate increases in the presence of oxygen.(Most bacteria)
   - ✓ *The pathway:*
     - Glucose →glycolysis (anaebobes)→ 2 Pyruvate +2ATP
     - No Kreb's cycle and other pathway (absence of carriers)
     - Result: accumulate acid and alcohol [fermentation]

4. Micro-aerophilic
   - ✓ These bacteria *do not grow in the presence* of high concentrations of oxygen because they produce excessive amounts of superoxide and hydrogen peroxide (H_2O_2).
   - ✓ Low O_2 (2-10% O_2): this percentage of O2 helps them manage reactive oxygen species.
   - ✓ E.g :*Campylobacter, Helicobacter*

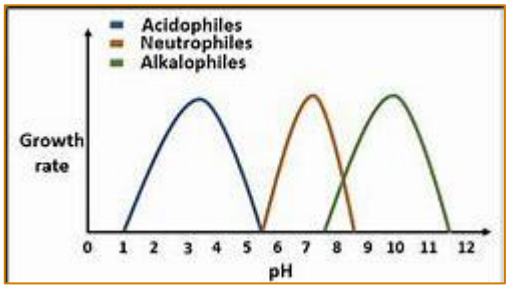
5. Aero-tolerant
   - ✓ *Do not require* oxygen for growth but can tolerate its presence because they produce a low concentration of superoxide dismutase.
   - ✓ E.g: *Cl.perfringens*

C) CO2 requirements

1. CO2 (0.03%) : Present in air is sufficient [most bacteria]
2. CO2 (5-10%) : Capnophilic
   - Neisseria
   - Brucella

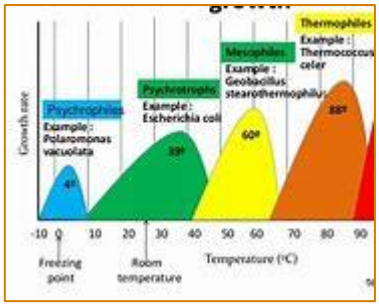
D) pH

- *Neutrophilic*: pH (7.2 – 7.4) (Most bacteria)
- *Alkalophilic*: pH (9) (*Vibrio cholerae*)
- *Acidophilic*: pH (4) (*Lactobacilli*)



E) Temperature

- *Mesophilic* (20 – 45) (Most bacteria)
- *Psychrophilic* (0 – 15)
- *Thermophilic* (55– 65)



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