Bacterial Growth & Physiology

Wednesday, October 30, 2024 6:20 PM

- **Bacterial Growth**: an increase in the size & number of an organism
  - → Indication / Detected by:
    1. Turbidity found in fluid growth media (when bacteria is inoculated & left for hours)
    2. Colonies appearing on solid media
       - ↳ Macroscopic products → bcz they're seen by naked eye

- **A Colony / Macroscopic Product**: the result of a single bacteria undergoing binary fission (20-30 divisions) to produce a visible mass of cells that appear as a dot/spot on solid media
  - → # of bacteria in a colony = 2^{20} = 1 million & 24 thousand (up to 2^{30})
    - ↳ the base is 2 bcz a single bacterium divides into 2 daughter cells

- **Generation / Doubling time**: the time it takes for a bacterial cell to divide
  - → some are fast: V. cholerae → 1 division every 13 mins
  - → some are slow: M. tuberculosis → 1 division every 24 hrs

- **Bacterial Reproduction**
  - ↳ by binary fission

Steps

1. Elongation → of the cell
   - * usually in one direction to give the daughter cells the same size as the parent cell
2. Seperation of DNA strands
   - ↳ each strand attaches to the mesosomes on opposite sides
     - ↳ which has enzymatic activity (like the mitochondria)
3. Each ssDNA becomes dsDNA
   - ↳ acts as a template for DNA replication to occur
4. Formation of Division Septum (aka. Septal Mesosome)
   - ↳ Keeps on constricting/narrowing until division ends (step 5)
5. Cell Seperation → 2 daughter cells of the same size & genetic material are produced

- **Culture Media**: a media that has the nutrients that the bacteria needs for growth in VITRO
  - ↳ may be artificial (proteins, salts, sugars, etc)
  - → Purposes:
    1. Study bacterial Properties
    2. Isolation, Identification & Diagnosis
       - * Causative Agents (is it bacteria? what type? etc)
    3. Selecting proper Antibiotics
    4. Prepare vaccines / Products
       - ↓ could be made from any bacterial components
       - ↳ E.g. Strepto pyogenes —gives→ StreptoKinase
         - ↳ given to heart attack survivors after purification to dissolve blood clots
  - → Classification
    - → liquid / broth —in→ test tubes
    - → semi Solid / jello —on→ petri dishes
  - → Types
    - → **Simple** (basic requirements)
      1. Peptone Water
         - [peptone + 0.5% NaCl]
         - Enhancement / Growth Support
         - A base for making Sugar Media
      2. Nutrient Broth
         - [Contains Meat Extract]
         - Enhancement
      3. Nutrient Agar Plate
         - [Nutrient broth + 2% Agar Agar] — [suitable for S. aureus]
         - ↳ Agar Agar → dried Seaweed
           - ↳ gets boiled w Nb then put in room temp → solidifies → jelly texture
    - → **Enriched** (for fasidious (needy) bacteria) — blood / serum
      1. Blood Agar
         - [Nutrient Agar (simple) —heat 45°C→ Add Sheep blood]
           - ↳ no antibiotics/antibodies that affect the results
         - [Suitable for: Streptococci (some that has hemolytic activity)]
         - ★ Hemolysis on blood agar (RBC breakdown)
           - (types)
             1. Complete (β-) hemolysis — Staph. aureus, Strepto. pyogenes = Clear area
             2. Partial (α-) hemolysis — Strepto. viridans, premonococci = green-ish area
             3. No (γ-) hemolysis — Enterococci = No color change (Red area)

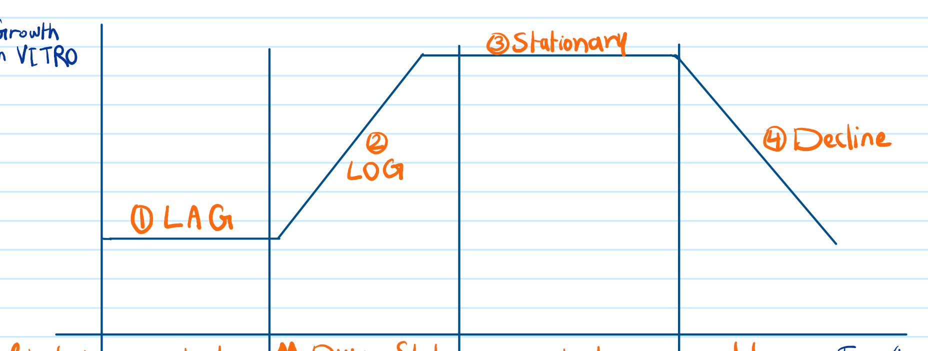
Aura of Pure Very Green Lungs is Entertaining (s. aureus, s. pyogenes, S. viridan, *hint, premono, Enterococci)

      2. Chocolate Agar
         - [Nutrient Agar (simple) —heat 100°C→ immediately Add Sheep blood]
           - ↳ Hemoglobin will breakdown
             - ↳ Haematin (dark, chocolaty color)
         - [suitable for: - Haemophilus - Neisseria]
    - → **Selective** (allows/supports a certain organism, inhibits others)
      - ↳ using dyes/Chemicals/Antibiotics
      1. Lowenstein Jensen Medium
         - [Selective ingredient: Malachite Green]
         - selective for [Mycobacterium tuberculosis]
      2. Blood Tellurite Agar
         - [Selective ingredient: Potassium Tellurite]
         - selective for [C. Diphtheriae]
    - → **Differential** — Selective + presence of an indicator
      - ↳ for differentiating bacteria by visible changes
      1. MacConkey's Agar
         - [Selective ingredient: Bile] —for→ [Enterobacteria]
         - others: Lactose (test sugar), Peptone, Neutral Red (pH indicator)
           - used to differentiate enterobacteria based on their ability to ferment lactose

         - if its a Lactose fermenter → pH↓, Acidic → Colonies ↝ Pink
         - Not a Lactose fermenter → x acid → Colonies ↝ Pale/Colorless
      2. Mannitol Salt Agar
         - [Selective ingredient: high Salt content 7.5% NaCl]
         - for [Staphylococci]
         - Phenol Red (pH indicator), Peptone, Mannitol (test sugar)
           - used to differentiate staphylococci based on their ability to ferment Mannitol

         - if its a Mannitol fermenter → ↓pH, Acid → Yellow (S. aureus)
         - NOT a Mannitol fermenter → x Acid → no color change/red (S.spp / S. epidermis)
      3. TCBS Agar (Thiosulfate - Citrate - Bile - Sucrose)
         - [Selective ingredient: Citrate, Bile]
         - for [Vibrio]
         - Bromothymol Blue (pH indicator), Sucrose (test sugar)
           - used to differentiate vibrio based on their ability to ferment Mannitol
         - if its a Mannitol fermenter → ↓pH, Acid → Yellow (Vibrio Cholerae)
         - NOT a Mannitol fermenter → x Acid → Green (other vibrio bacteria)

- **Bacterial Growth Curve** (in VITRO): 4 phases that occur as a cycle when a small number of bacteria is inoculated in a liquid nutrient medium & observed for 72 hrs



	① LAG	② LOG	③ Stationary	④ Decline
# of bacteria	constant	↑↑, Division Starts	constant ↳ Division = Death	↓↓ ↳ increased death
Protein Syn	↑↑ to adapt to the new medium w ↑ nutrients	↑↑	—	↓↓ ↳ bcz of Nutrient/O_2 depletion
Size	(result) ↑↑	↓ goes down compared to Log bcz of cell division but is still larger than other phases	—	
★ AKA	* Adaptation Phase *	* Golden Phase *	—	
Notes		* Antibiotics action occurs in this phase ↳ to inhibit growth	Nutrients start to deplet, Bacterial waste products start to accummulate	Waste Products accummulation is toxic
Equivelant to human	(Incubation Phase)	(Invasive Phase) symptoms of infection occur	(Course of Disease) start & end of disease	(Convalescent / Recovery Phase)

- → When Bacterial Growth Curve (which is in VITRO) is applied to humans when bacteria grows inside them to cause infections (in VIVO) the names of the phases change

Bacterial Growth Requirements

- → ① **Nutrition**
  1. Autotrophic Bacteria (self - nutrition)
     - * Utilizes Simple Inorganic Subs
       - CO_2 ↓ source of C; NH_4^+ ↓ source of N → complex organic material
     - Saprophytic Bacteria — found in soil, air, etc

     - * No medical Significance (Doesn't Cause Diseases)
  2. Heterotrophic (different)
     - ↳ many modes of nutrient supply
     - * Requires Complex Organic Material (Protein, Sugars etc) from a living host
       - ↳ Parasytic *
         - ↳ * Medically Significant ✓
- → ② **Gaseos**
  - → ① O_2 Requirements
    - Bacterial Respiration: the Glucose catabolism done for Energy Production
    1. Obligate Aerobic — O_2: Present = Growth ✓ / Absent = No Growth ✗
       - E.g. Pseudomonas aeruginosa
       - ↳ Glycolysis = 2ATP, 2NADH
       - ↳ Krebs = 2ATP, 8NADH, 2FADH2
       - ↳ OxiPhos = 34 ATP — $2H^+ + 2e^-$ gets carried in O_2
       - [38 ATP] in total
         - ↳ Large amount of energy ✓
       - * When O_2 is in OxiPhos → Toxic material is produced: O_2^- (superoxide), H_2O_2 (Peroxide)
         - ↓ SuperOxide Dismutase, ↓ Catalase — Obligate Aerobics have them in large quantities
           - ↳ these enzymes metabolize the toxins
    2. Obligate Anaerobic — O_2: Present = No Growth ✗ / Absent = Growth ✓
       - E.g. Bacteroids fragilis
       - ↳ Glycolysis = 2ATP, 2NADH
       - ↳ Krebs = 2ATP, 8NADH, 2FADH2
       - ↳ alternative Pathway → inorganic molecules carry H^+/e^- [Nitrate, Sulfate, CO_2]
         - ↳ Prod 13 ATP
       - [∴ tot = 17 ATP]
       - this bacteria type Lacks SOD, Cat → even if O_2 was present & it went through OxiPhos → cell will die by excess toxic material
    3. Faculative Anaerobic — O_2: Present = ↑↑↑ Growth ✓ / Absent = Growth ✓
       - E.g. Most Bacteria
       - ↳ Glycolysis = 2ATP, 2NADH
       - ↳ Fermentation *
       - in case of O_2 Absence: G —glycolysis→ Pyruvate + [2 ATP]; Ferment → Alcohol & Acid (regenerate NAD+)
       - in case of O_2 presence: Glycolysis → Krebs → OxiPhos — Similar to Obligate Aerobic
       - No Carries → x Krebs x Alternative Pathways
    4. Micro - aerophilic — O_2: Present = No Growth ✗ / LOW = Growth ✓ / Abscent = Growth ✗
       - E.g. Campylobacter, Helicobacter
       - * Has LOW superoxide dismutase & Catalase
       - in case of high O_2: ↑O_2 = ↑toxins BUT ↓enz → bacteria Dies = No Growth
       - in case of Low O_2: ↓O_2 = ↓toxins & ↓enz → bacteria Growth ✓ (glycolysis ↳ Krebs ↳ OxiPhospho)
       - in case of No O_2: no carriers → x alt pathways; no O_2 → x oxiphospn ∴ No Growth
    5. Aero-tolerant Anaerobes — O_2: Present = No Growth ✗ / LOW = Tolerate * / Abscent = Growth ✓
       - E.g. Cl. perfringes
       - * Has superoxide dismutase, Does Not have catalase (↳ H_2O_2)
       - in case of high O_2: ↑O_2 = ↑H_2O_2 BUT ↓enz → bacteria Dies = No Growth
       - [↓enz ↝ only ① one]
       - in case of Low O_2: ↓O_2 = ↓H_2O_2 & ↓enz → tolerate
       - in case of No O_2: xO_2 — x carriers for anaerobic respiration ✓ fermentation ↳ = Growth

"Pseudo Bacteria Frequently Camp High Places" (O.aero, O.anaero, Faculative = most, micro-aero, Aero tolerant)

  - → ② CO_2 Requirements
    - 0.03% CO_2 ↳ present in air — [Most Bacteria]
    - 5-10% CO_2 for Capnophilic bacteria — [Neisseria, Brucella]
- → ③ **pH & Temperature**
  - pH
    1. Neutral — pH ~ 7.2 - 7.4 — Bactera that prefers this pH is called neutrophilic — [Most Bacteria]
    2. Acidic — pH ~ 4 — Acidophilic — [Lactobacilli]
    3. Alkaline — pH ~ 9 — Basophilic — [Vibrio Cholerae]
  - Temp
    - ↳ Mesophilic 20 ~ 45 °C — [Most bacteria]
    - ↳ Psychrophilic 0-15 °C
    - ↳ Thermophilic 55 - 65 °C

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