Microbial Growth

Chapter 6

Microbial Growth - refers to the # of cells, not the size of the cells

- Requirements for Growth
  - Physical
  - Chemical

Physical Requirements

- Temperature
  - psychrophiles (cold loving microbes)
    - range 0 C - 20 C
  - mesophiles (moderate temp. loving microbes)
    - range 20 C - 40 C
  - thermophiles (heat loving microbes)
    - range 40 C - 100 C

pH

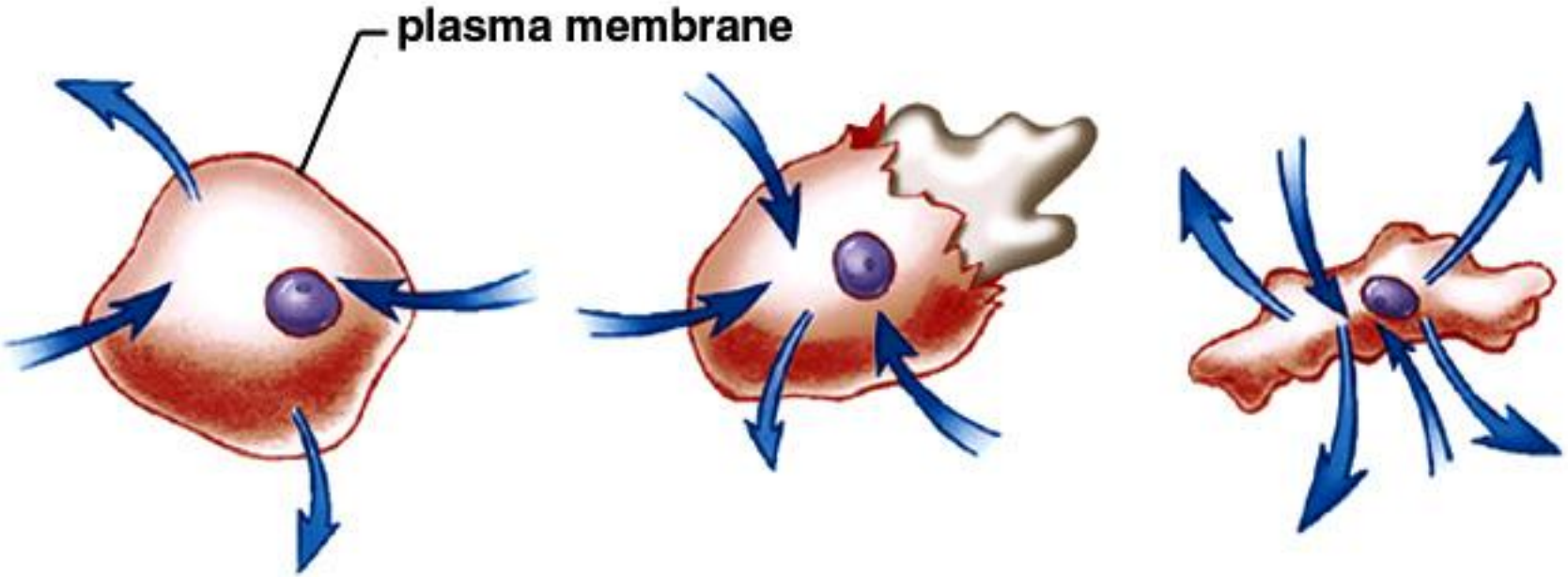
- Most bacteria grow between pH 6.5 - pH 7.5
- Very few can grow at below pH 4.0
  - many foods, such as sauerkraut, pickles, and cheeses are preserved from spoilage by acids produced during fermentation

Osmotic Pressure

- Microbes obtain almost all their nutrients in solution from surrounding water

- Tonicity
  - isotonic
  - hypertonic
  - hypotonic

Cells



Animal Cells

Chemical Requirements

- Macro & Micro Elements

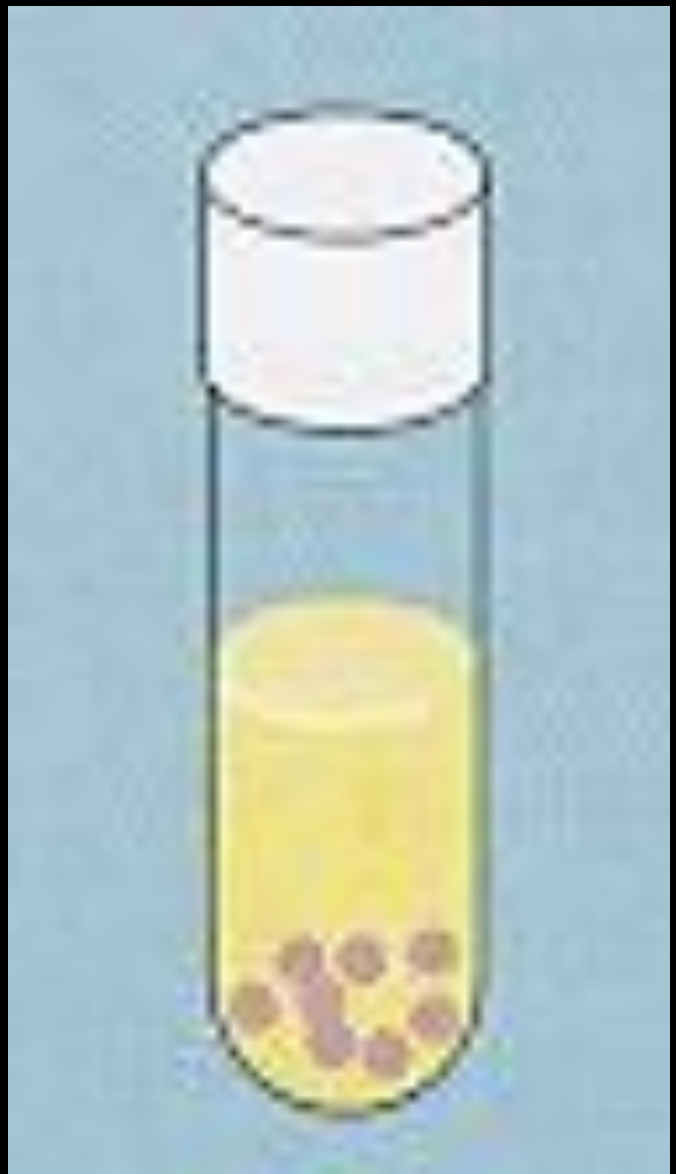
- C HOPKINS CaFe Mg NaCl

Oxygen

- Bacteria can be classified base on their oxygen requirements

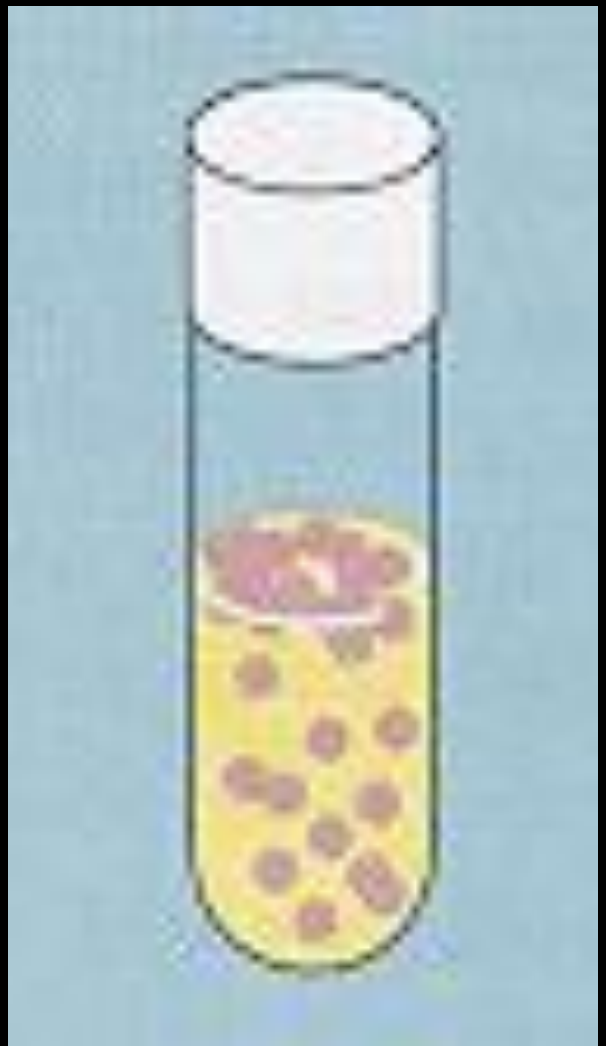
1. Obligate Aerobes

2. Obligate Anaerobes

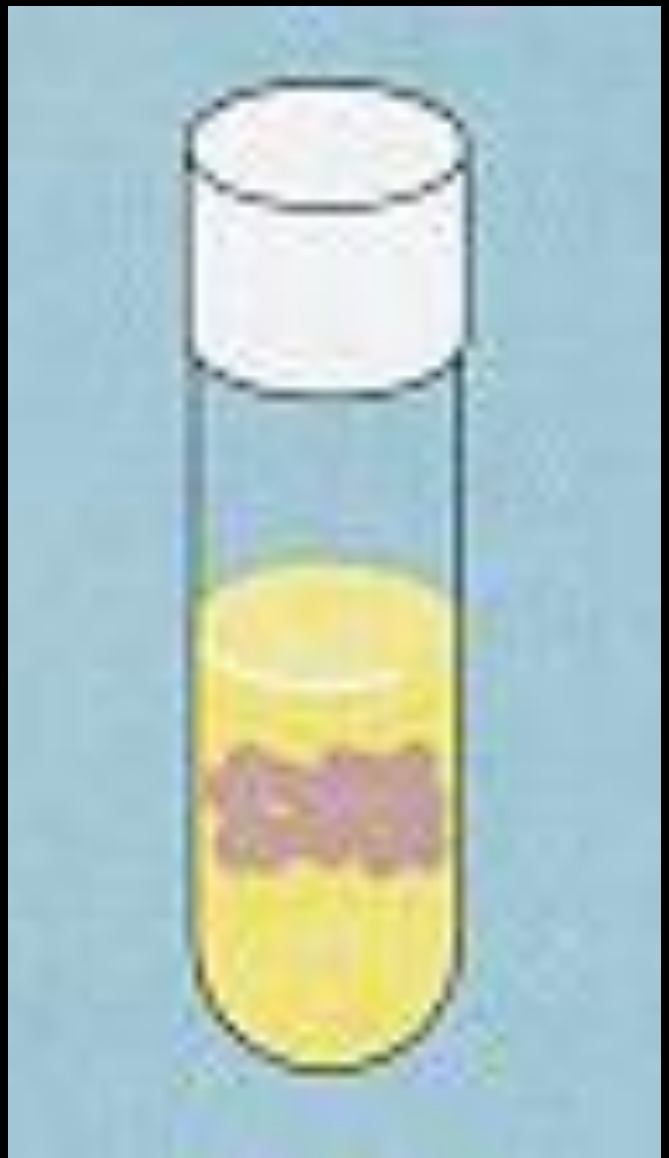


3. Facultative Aerobes

Facultative Anaerobes



4. Microaerophilic



Oxygen is lethal to some organisms

- All organisms produce superoxide (O_2-)
- Superoxide is toxic to cells (steals electrons)
- Superoxide must be neutralized

Superoxide dismutase

- O_2^- + O_2^- + 2 H^+ -------> H_2O_2 + O_2
- Hydrogen peroxide is also toxic to cells and it must be neutralized

Catalase

- $2\ H_2O_2 \text{ --------> } 2\ H_2O + O_2$
- Obligate Anaerobes lack:
  - Superoxide dismutase (SOD)
  - Catalase

Culture Media

- 1. Chemically Defined
  - the exact chemical composition is known
  - used to grow fastidious organisms
- 2. Complex Media
  - exact chemical composition is not known
  - most bacteria and fungi are grown with this

Special Culture Techniques

- 1. Anaerobic Bacteria
  - a. Reducing Media
  - b. Anaerobic Container
  - c. Agar Stab
  - d. Agar Shake

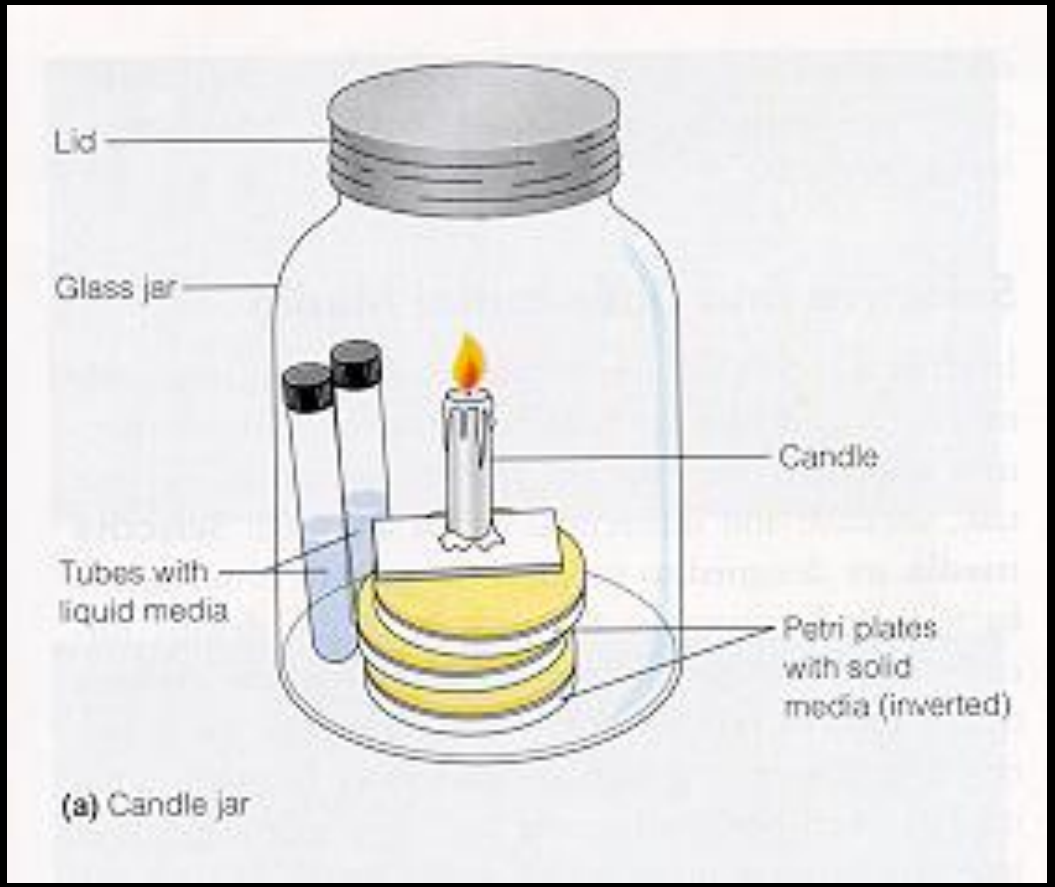
Special Culture Techniques

- 2. Microaerophilic Bacteria
  - grow best under reduced O_2 levels and increased CO_2 levels

  - Normal Atmosphere 21 % O_2
  - .3 to .03 % CO_2

Microaerophilic Bacteria

A. Candle Jar

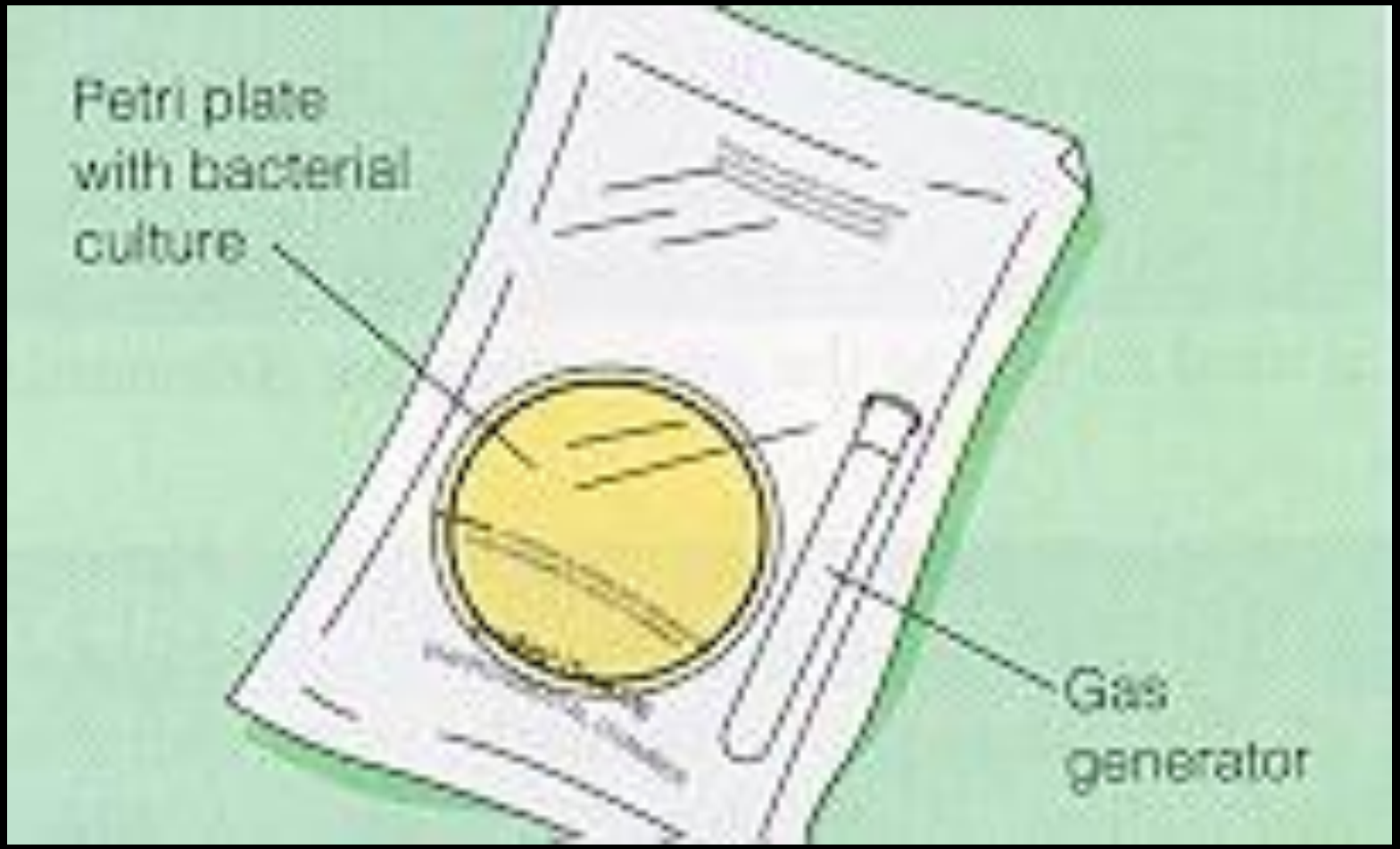


16 % O_2

4 % CO_2

Microaerophilic Bacteria

B. CO_2 Generating Packet



Selective Media

- Inhibits the growth of some bacteria while selecting for the growth of others
- Example:
  - Brilliant Green Agar
    - dyes inhibit the growth of Gram (+) bacteria
    - selects for Gram (-) bacteria
    - Most G.I. Tract infections are caused by Gram (-) bacteria

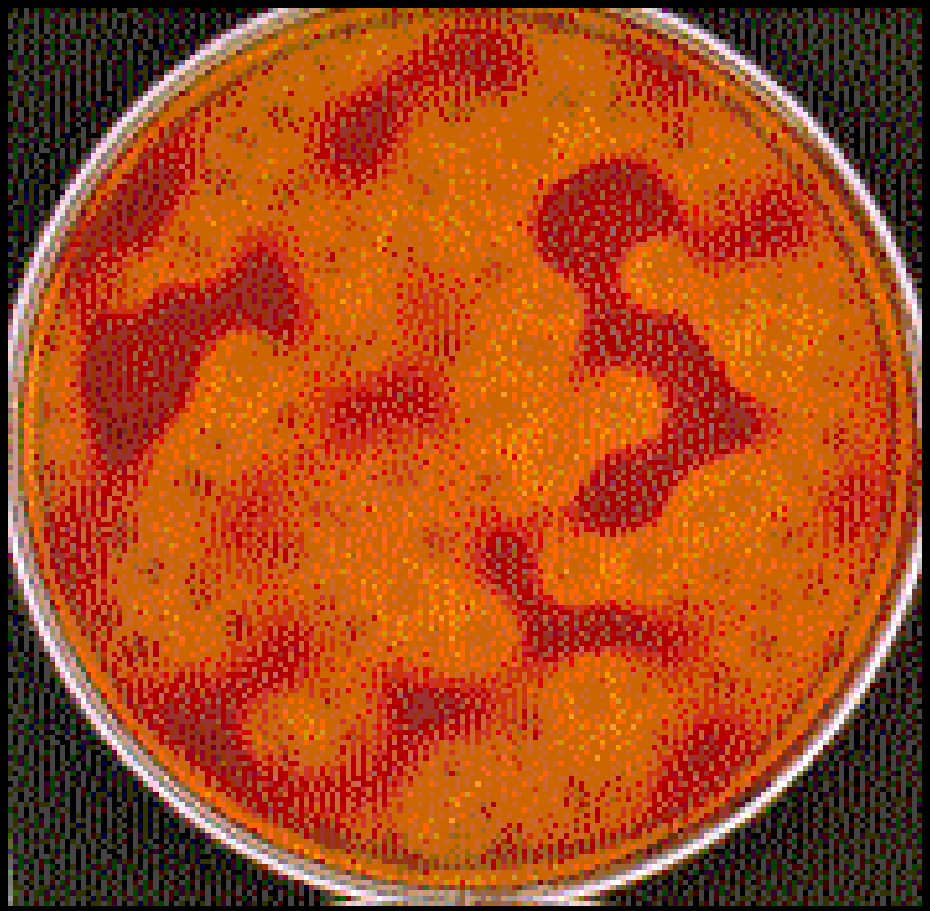
Selective Media

- EMB (Eosin Methylene Blue)
  - dyes inhibit Gram (+) bacteria
  - selects for Gram (-) bacteria
  - G.I. Tract infections caused by Gram (-) bacteria

Differential Media

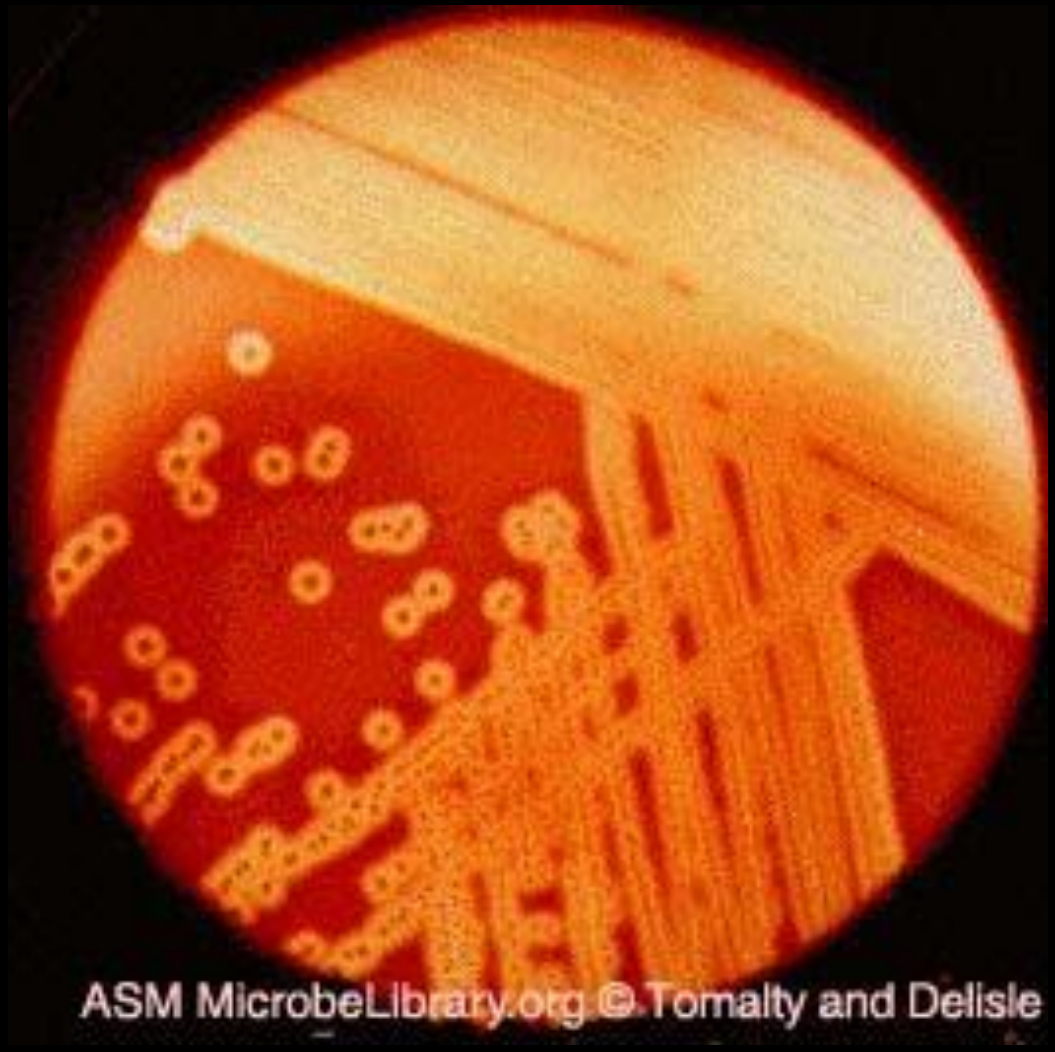
- Differentiates between different organisms growing on the same plate
- Example:
  - Blood Agar Plates (TSA with 5% sheep blood)
    - used to differentiate different types of *Streptococci*

Alpha Hemolytic *Streptococci*



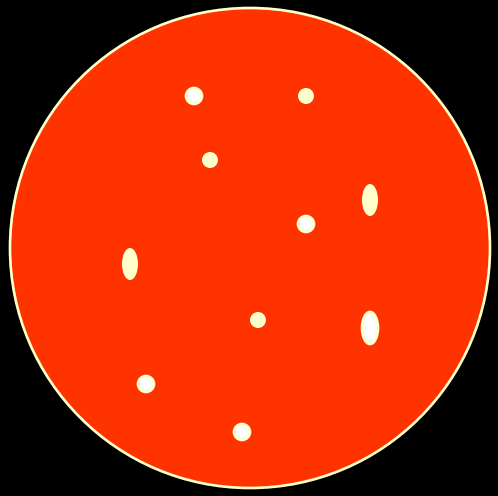
Incomplete lysis of RBC's

Beta Hemolytic *Streptococci*



Complete lysis of RBC's

Gamma Hemolytic *Streptococci*



No lysis of RBC's

Selective and Differential Media

- Mannitol Salt Agar
  - used to identify *Staphylococcus aureus*
- Mannitol Salt Agar
  - High salt conc. (7.5%) inhibits most bacteria
  - sugar Mannitol
  - pH Indicator (Turns Yellow when acid)

Selective and Differential Media

- MacConkey's Agar
  - used to identify *Salmonella*
- MacConkey's Agar
  - Bile salts and crystal violet (inhibits Gram (+) bacteria)
  - lactose
  - pH Indicator

Many Gram (-) enteric non-pathogenic bacteria can ferment lactose, *Salmonella* can not

Bacterial Growth - increase in the # of cells

- Binary Fission

- Generation Time (Doubling Time)
  - time required for a cell to divide
  - most about 1 Hr. To 3 Hrs.
    - *E. coli* - 20 minutes
    - *Mycobacterium tuberculosis* - 24 Hrs.

Binary Fission - unchecked

- E. coli - generation time of 20 min.
- 20 generations (about 7 hrs.)
  - 1 million cells
- 30 generations (about 10 hrs.)
  - 1 billion cells
- 72 generations (about 24 hrs.)
  - 1×10^{21}
    - 1,000,000,000,000,000,000,000 cells

Limiting factors in the environment

- Lack of food, water or nutrients
- space
- accumulation of metabolic wastes
- lack of oxygen
- changes in pH
- temperature

Phases of Growth

- 4 Phases

- 1. Lag Phase
- 2. Log Phase
- 3. Stationary Phase
- 4. Death Phase

1. Lag Phase

- Bacteria are first introduced into an environment or media
- Bacteria are “checking out” their surroundings
- cells are very active metabolically
- # of cells changes very little
- 1 hour to several days

2. Log Phase

- Rapid cell growth (exponential growth)
- population **doubles** every generation
- microbes are sensitive to adverse conditions
  - antibiotics
  - anti-microbial agents

3. Stationary Phase

- Death rate = rate of reproduction
- cells begin to encounter environmental stress
  - lack of nutrients
  - lack of water
  - not enough space
  - metabolic wastes
  - oxygen
  - pH

Endospores would form now

4. Death Phase

- Death rate > rate of reproduction
- Due to limiting factors in the environment

Enumeration of Bacteria

- Turbid culture - 10 million bacterial cells per ml
- Serial Dilution