*PowerPoint® Lecture Slides for*

# MICROBIOLOGY

ROBERT W. BAUMAN

## Chapter 6

# Microbial Nutrition and Growth

# Definitions

- **Microbial growth** – an increase in number of cells (microbes), not in cell size
- _____ – an aggregation of cells arising from single parent cell
- Metabolism gives the cell the _____ for reproduction
- Reproduction results in growth

# Growth Requirements: Physical

- Physical Requirements
  - Temperature
  - pH
  - _____ Pressure
  - Hydrostatic Pressure

# Temperature

- Effect of temperature on proteins
- Effect of temperature on lipid-containing membranes of cells and organelles
  - If too low, membranes become _____ and fragile
  - If too high, membranes become too fluid and cannot contain the cell or organelle

# Effects of Temperature on Growth

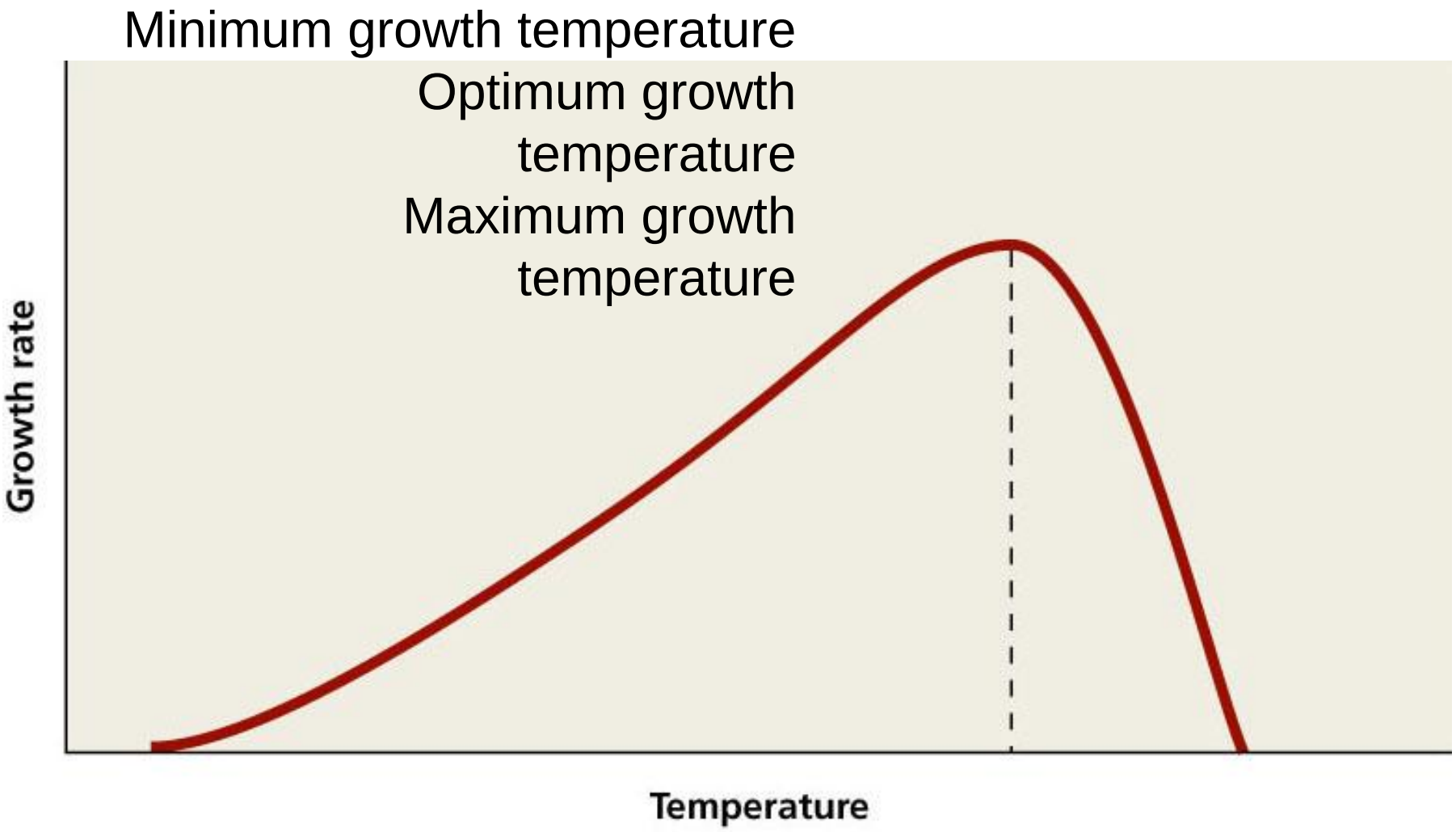



Figure 6.4

# Effects of Temperature on Growth

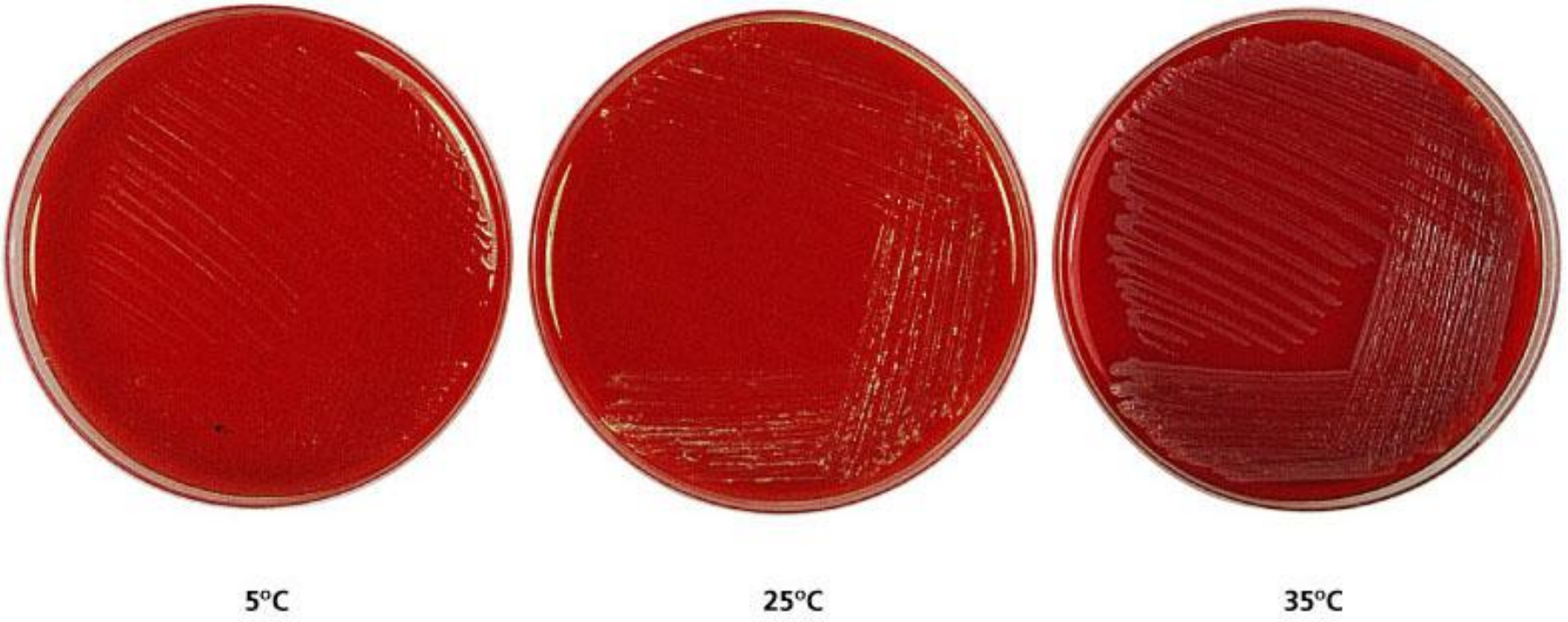



Figure 6.4

# Catagories of Microbes Based on Temperature Range

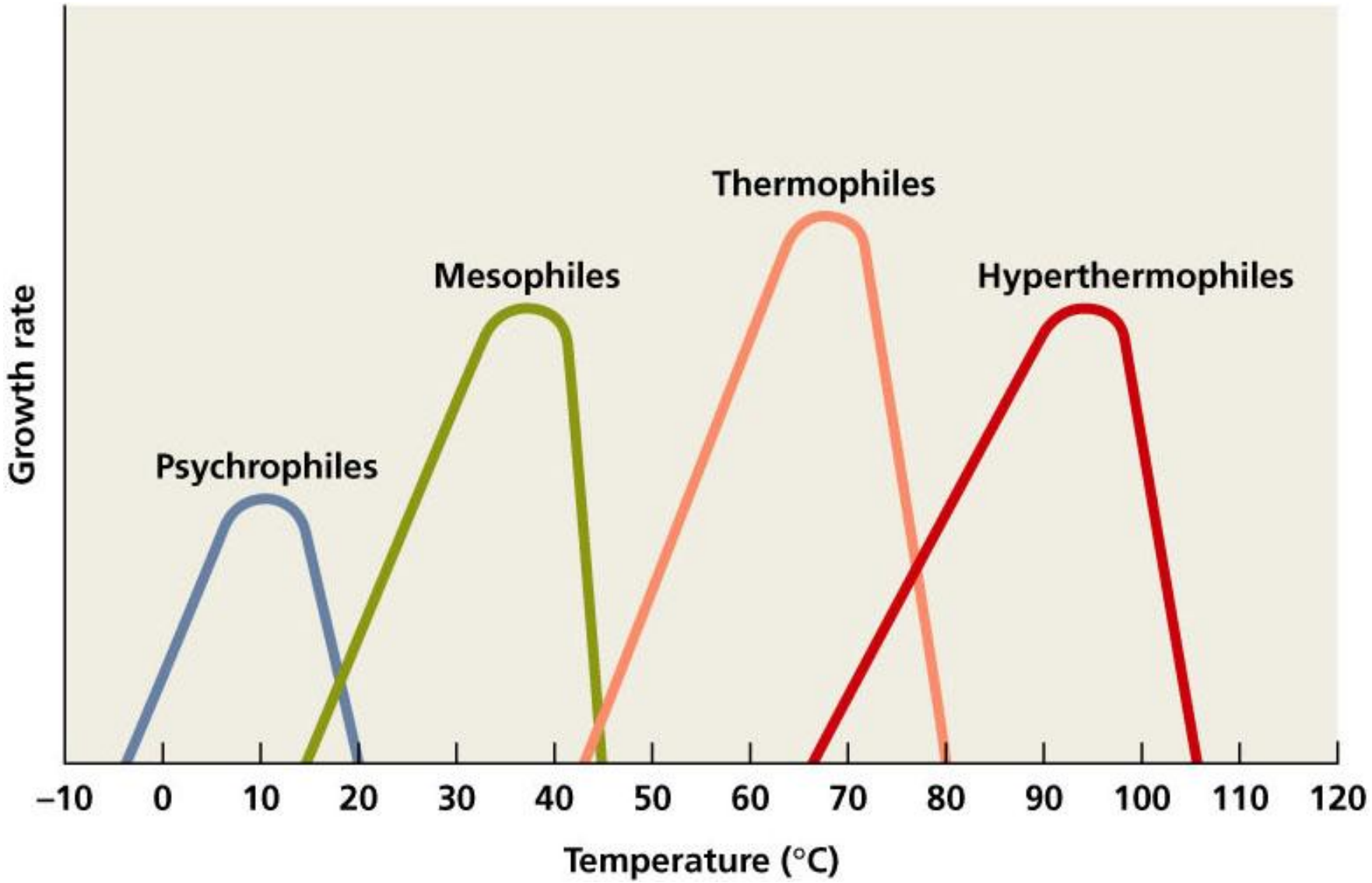



Figure 6.5

# Psychrophiles

Grow between 0°C - 20°C
Cause food spoilage

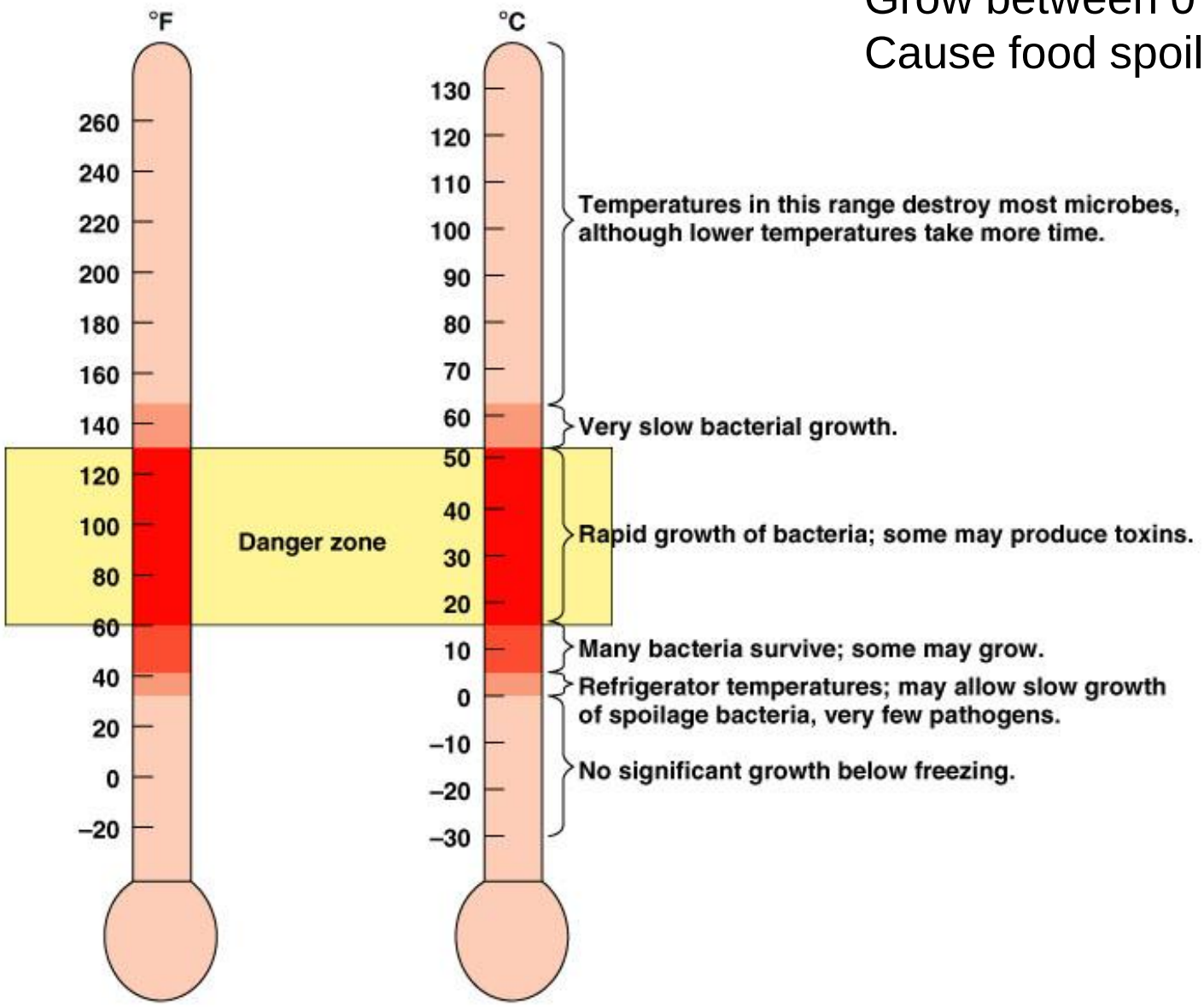





Figure 6.2

# The Requirements for Growth: Physical Requirements

- pH
  - $H^+$ and $OH^-$ interfere with H bonding in proteins and nucleic acids
  - Most bacteria & protozoa grow between pH 6.5 & 7.5
    - _____
  - **Acidophiles** grow in acidic environments
    - Molds and yeasts grow between pH 5 and 6
  - **Alkalinophiles** live in basic environments (soils & water) pH 9 to pH 11.5

# The Requirements for Growth: Physical Requirements

- **Water Requirements**
- **Osmotic Pressure** – Pressure exerted on a semi-permeable membrane by a solution containing solutes that cannot freely cross membrane.
  - related to concentration of dissolved molecules and ions in a solution
  - Metabolic reactions take place in water
  - Most cells die in absence of water

# The Requirements for Growth: Physical Requirements

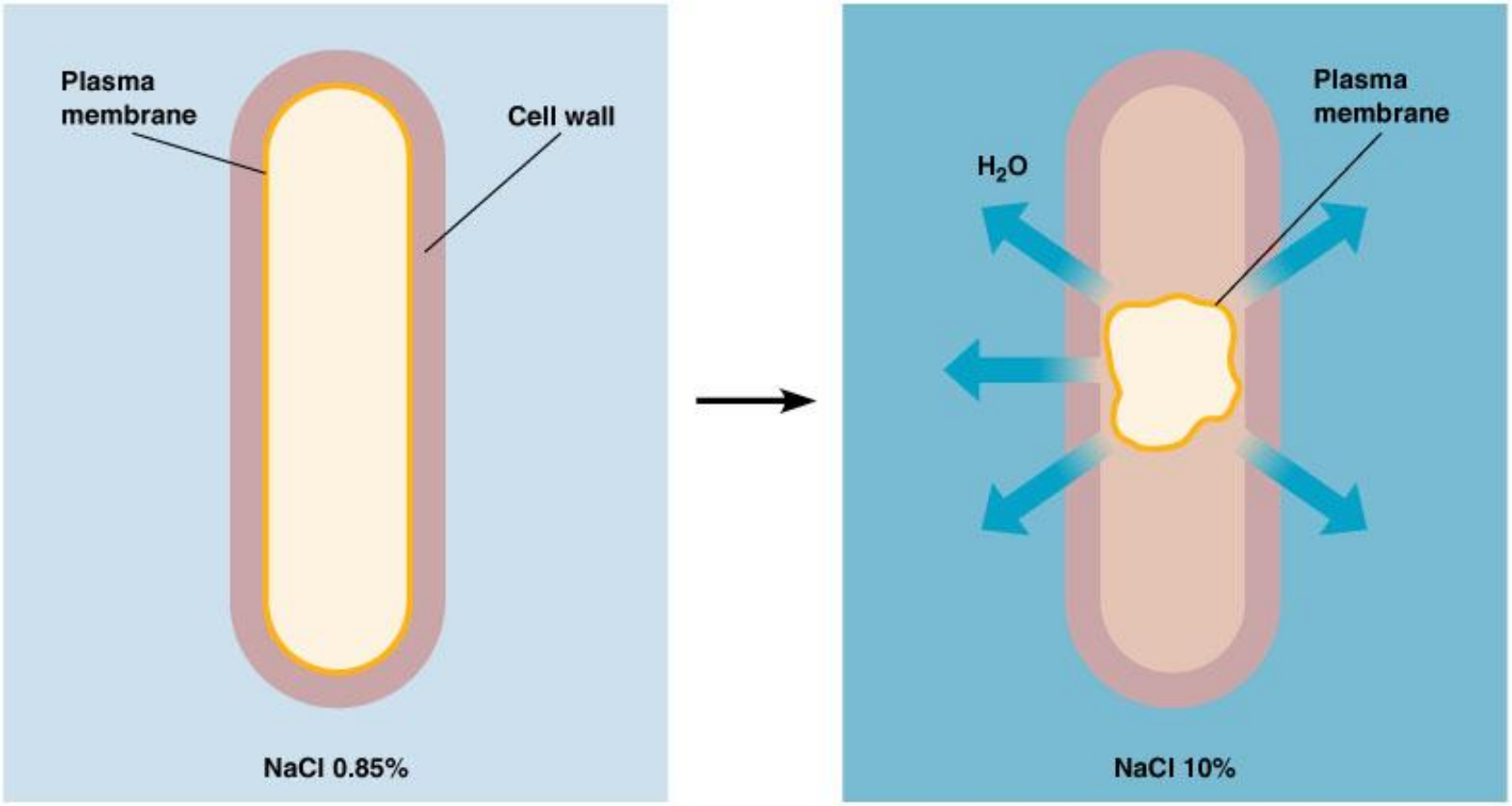





Figure 6.4

# Physical Effects of Water

- **Hypertonic environments -**[solute] higher or lower?
  - increased salt or sugar → plasmolysis
  - Extreme or *obligate* _____ require high osmotic pressure – up to 30% salt
  - *Facultative* halophiles tolerate high osmotic pressure
- **Hypotonic solutions -** lower solute concentrations
  - cells will swell and burst

# Hydrostatic Pressure

- Water exerts pressure in proportion to its depth
  - For every addition of _____, water pressure increases 1 atm
- Organisms that live under extreme pressure are **barophiles**
  - Their membranes and enzymes depend on this pressure to maintain their three-dimensional, functional shape

# Growth Requirements – Chemical Requirements

- Nutrients for
  - energy needs
  - to build _____ molecules
  - to build cellular structures
- Most common nutrients have the following elements –
  - _____?
- Microbes obtain nutrients from variety of sources

# The Requirements for Growth: Chemical Requirements

- **Carbon**
  - Structural organic molecules, energy source
  - Chemoheterotrophs use organic carbon sources
  - Autotrophs use $CO_2$
- **Nitrogen**
  - In amino acids, proteins & _____
  - Most bacteria decompose proteins
  - Some bacteria use $NH_4^+$ or $NO_3^-$
  - A few bacteria use $N_2$ in nitrogen fixation

# The Requirements for Growth: Chemical Requirements

- **Sulfur**
  - Some amino acids - disulfide bonds (protein ___ structure level?)
  - In vitamins - thiamine, biotin
  - Most bacteria decompose proteins
  - Some bacteria use $SO_4^{2-}$ or $H_2S$
- **Phosphorus**
  - In _____, ATP, and phospholipid membranes
  - $PO_4^{3-}$ is a source of phosphorus

# The Requirements for Growth: Chemical Requirements

- **Trace Elements**
  - Inorganic elements required in small amounts
  - Usually as enzyme cofactors

- **Growth factors** – organic chemicals that cannot be synthesized by certain organisms

# Sources of Carbon & Energy

- Organisms categorized into **two groups** based on **source of carbon**
  - **Autotrophs -** Those using an inorganic carbon source (carbon dioxide).
  - **Heterotrophs -** Those catabolizing reduced organic carbon molecules (proteins, carbohydrates, amino acids, and fatty acids).

# Sources of Carbon & Energy

- Organisms categorized into **two groups** based on whether they use chemicals or light as **source of energy**
  - **Chemotrophs -** Those that acquire energy from redox reactions involving inorganic and organic chemicals.
  - **Phototrophs -** Those that use light as their energy source.

# Four Basic Groups of Organisms

| | | Energy Source: Light (*photo-*) | Energy Source: Chemical compounds (*chemo-*) |
|---|---|---|---|
| Carbon Source | Carbon dioxide (*auto-*) | ***Photoautotrophs***<br>• Plants, algae, and cyanobacteria use $H_2O$ to reduce $CO_2$, producing $O_2$ as a byproduct<br>• Photosynthetic green sulfur and purple sulfur bacteria do not use $H_2O$ nor produce $O_2$ | ***Chemoautotrophs***<br>• Hydrogen, sulfur, and nitrifying bacteria |
| | Organic compounds (*hetero-*) | ***Photoheterotrophs***<br>• Green nonsulfur and purple nonsulfur bacteria | ***Chemoheterotrophs***<br>• Aerobic respiration: most animals, fungi, and protozoa, and many bacteria<br>• Anaerobic respiration: some animals and bacteria<br>• Fermentation: some bacteria and yeasts |

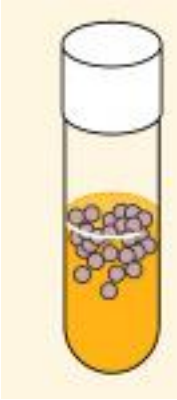
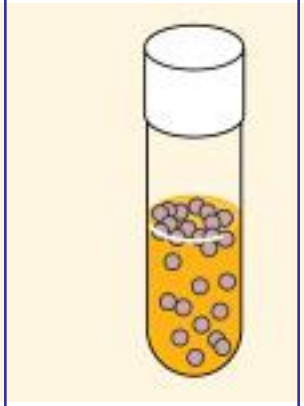
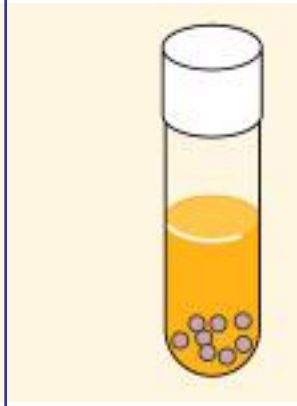
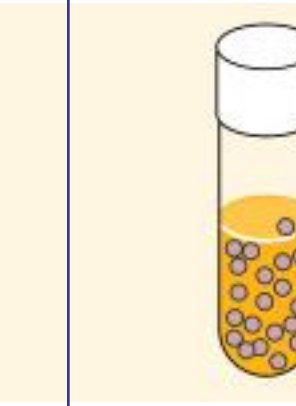
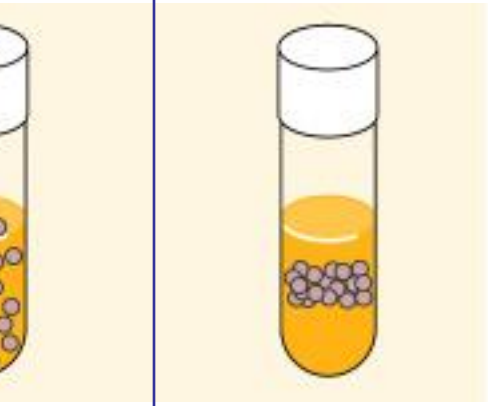
Figure 6.1

# Oxygen Requirements

- Organisms need and tolerate oxygen to different degrees
- Because of this they are classified into the following groups:
  - **Obligate Aerobes** – require oxygen → undergo aerobic respiration
  - **Microaerophiles** – aerobes that require lower levels of oxygen
    - levels from 2-10%
    - have a limited ability to detoxify hydrogen peroxide and superoxide radicals
  - **Facultative Anaerobes** – can maintain life via fermentation or anaerobic respiration or by aerobic respiration
  - **Aerotolerant anaerobes** – do not use aerobic metabolism but have some enzymes that detoxify oxygen's poisonous forms
  - **Obligate Anaerobes** – do not use aerobic metabolism → oxygen is toxic to them

# The Requirements for Growth: Chemical Requirements

- Oxygen ($O_2$)

| obligate aerobes | Faultative anaerobes | Obligate anaerobes | Aerotolerant anaerobes | Microaerophiles |
|---|---|---|---|---|
|  |  |  |  |  |

# Oxygen Reguirements

- Oxygen is essential for obligate aerobes (final electron acceptor in ETC)
- Oxygen is deadly for obligate anaerobes
- How can this be true?
  - Gaseous $O_2$ and oxygen covalently bound in compounds is not poisonous
  - The forms of oxygen that are _____ are excellent oxidizing agents
    - Resulting chain of oxidations causes irreparable damage to cells by oxidizing compounds such as proteins and lipids

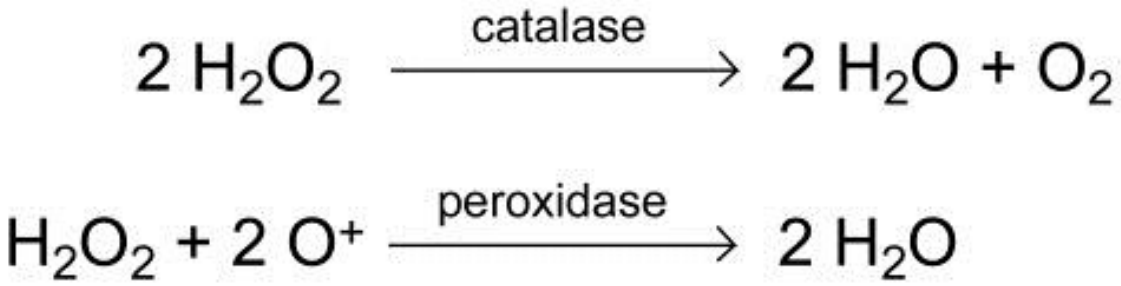
# Toxic Forms of Oxygen

- **Singlet oxygen: $O_2$** with electrons boosted to a higher-energy state
  - Occurs during photosynthesis → carotenoids
- **Superoxide free radicals: $O_2^-$**
  - formed during incomplete reduction of oxygen in aerobic and anaerobic respiration
  - So reactive that _____ produce superoxide dismutases to detoxify them
  - Anaerobes lack superoxide dismutase → die in the presence of oxygen

$$O_2^- + 2\,H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

# Toxic Forms of Oxygen

- **Peroxide anion: $O_2^{2-}$**
  - formed during reactions catalyzed by superoxide dismutase and other reactions
  - Aerobes contain either _____ or peroxidase to detoxify peroxide anion
  - Obligate anaerobes??




Where does this occur in an Eukaryotic cell?

# Toxic Forms of Oxygen

- **Hydroxyl radical (OH•)**
  - results from *ionizing radiation* and from incomplete reduction of hydrogen peroxide
  - The _____ of the four toxic forms of oxygen
  - Not a threat to aerobes due to action of catalase and peroxidase
- Vitamins for aerobes

# Nitrogen Requirements

- **Anabolism** often ceases due to **insufficient nitrogen**
  - Nitrogen is needed for **proteins** and **nucleotides**
- Nitrogen acquired from organic and inorganic nutrients
  - all cells recycle nitrogen from amino acids & nucleotides
  - In what subunit of a nucleotide would you find nitrogen?
- **Nitrogen fixation**
  - $N_2$ → ammonia (reduction)
    - Makes nitrogen available in a usable form
  - Preformed by certain bacteria
  - Essential to life on Earth.

# Culturing Microorganisms

- **Culture -** Microbes growing in/on culture medium
- **Inoculum -** Introduction of microbes into medium
- **Culture Medium -** Nutrients prepared for microbial growth
- **Sterile -** _____

# Obtaining Pure Cultures

- **Cultures** composed of cells arising from a single progenitor
- A **pure culture** contains only one species or strain
- **Colony -**
  - A colony is often called a colony-forming unit (CFU)
- **Aseptic technique -** used to prevent contamination of sterile substances or objects
  - And _____?
- Two common isolation techniques
  - Streak Plates
  - Pour Plates

# Streak Plate Method

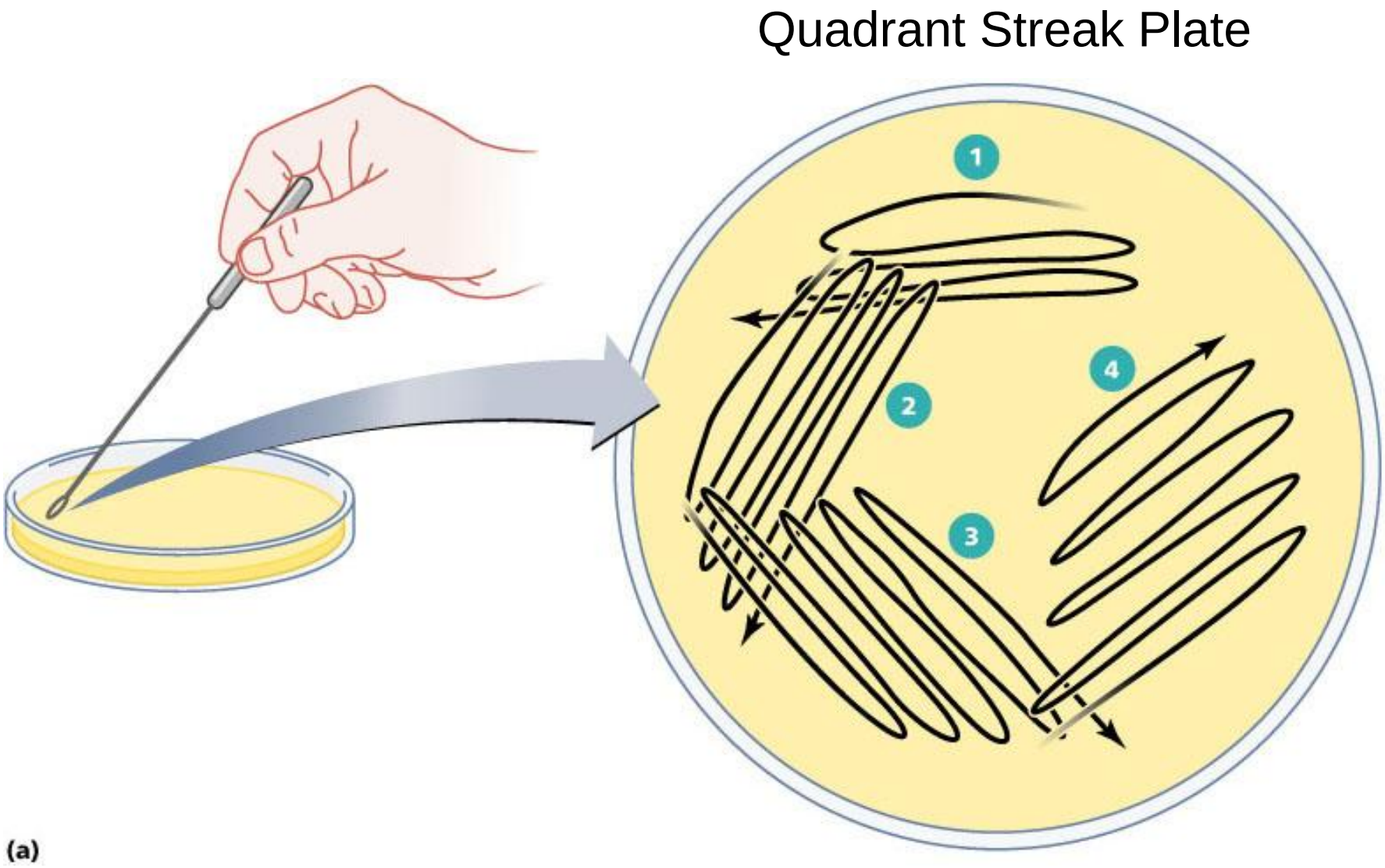
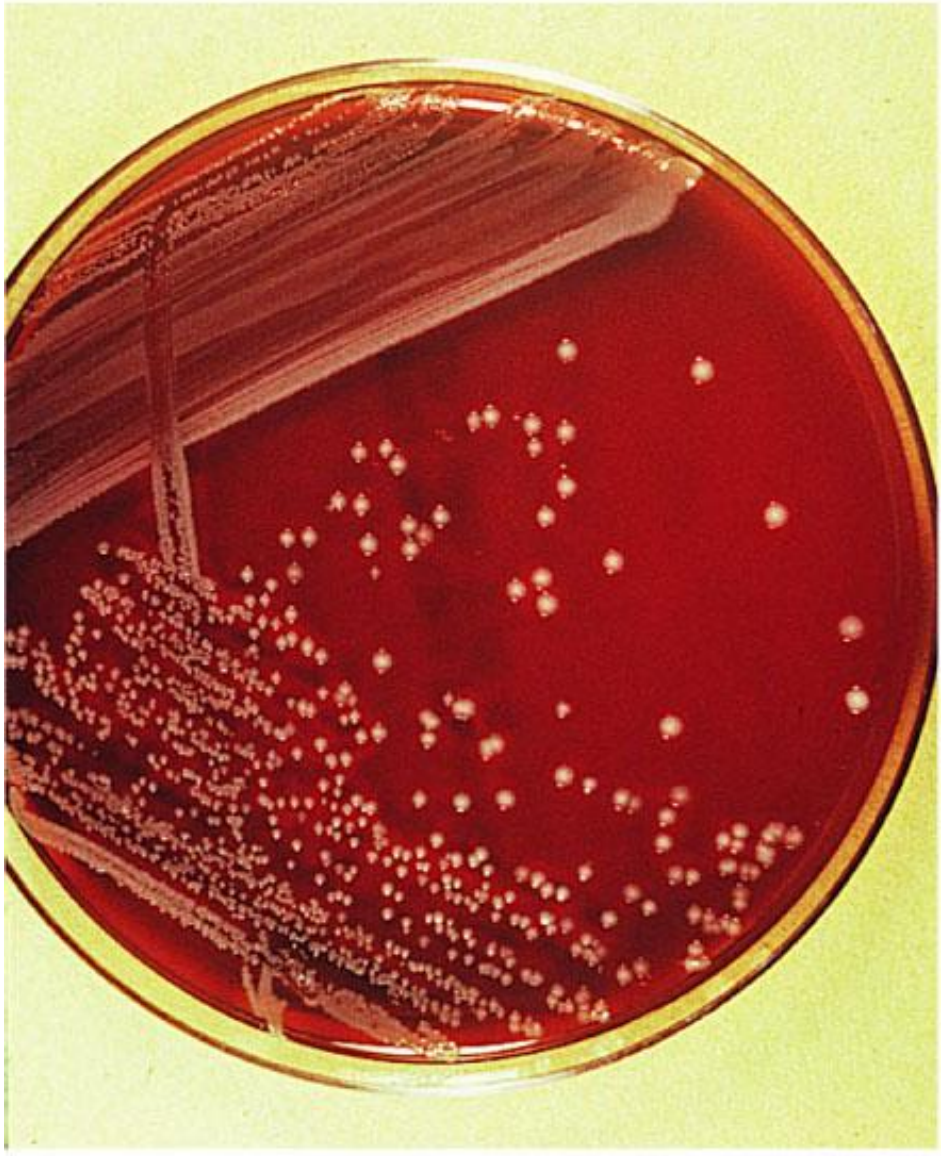



Figure 6.8

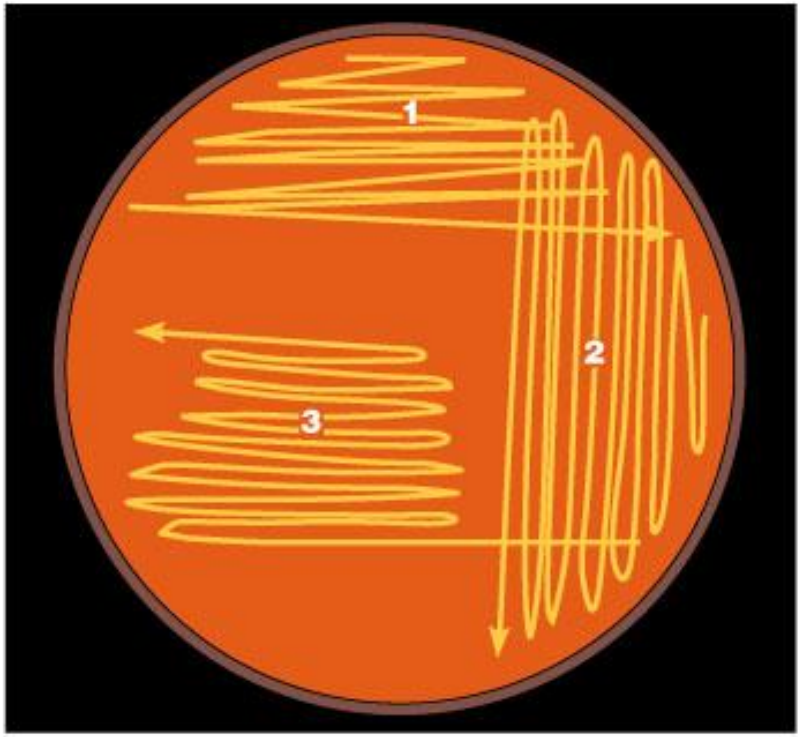
# Streak Plate Method



(b)

Figure 6.8

# Streak Plate



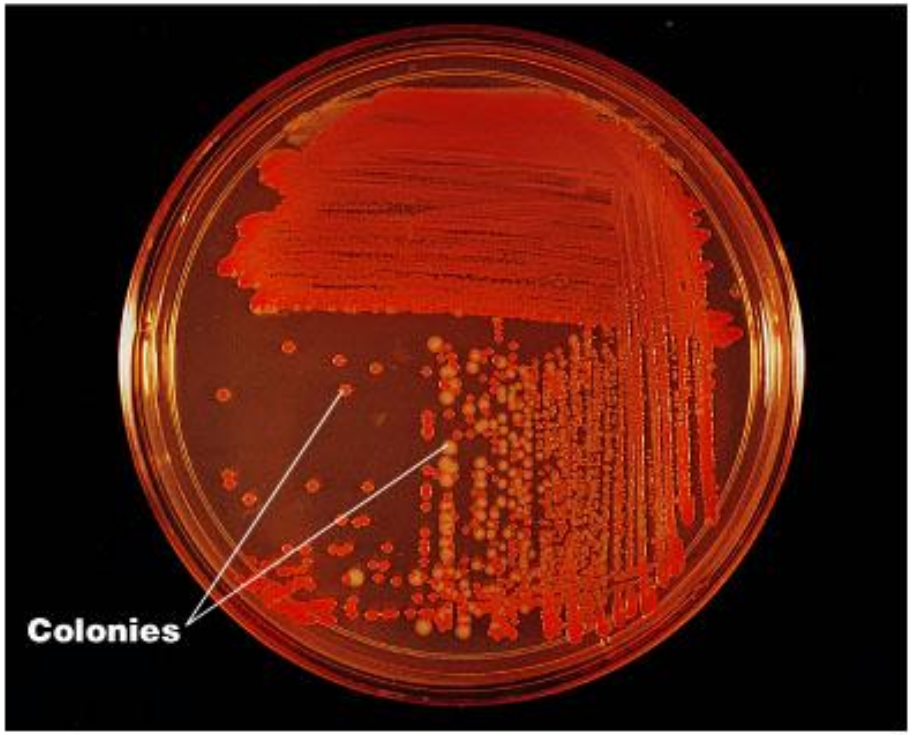






Figure 6.10a, b

# Pour Plate Method

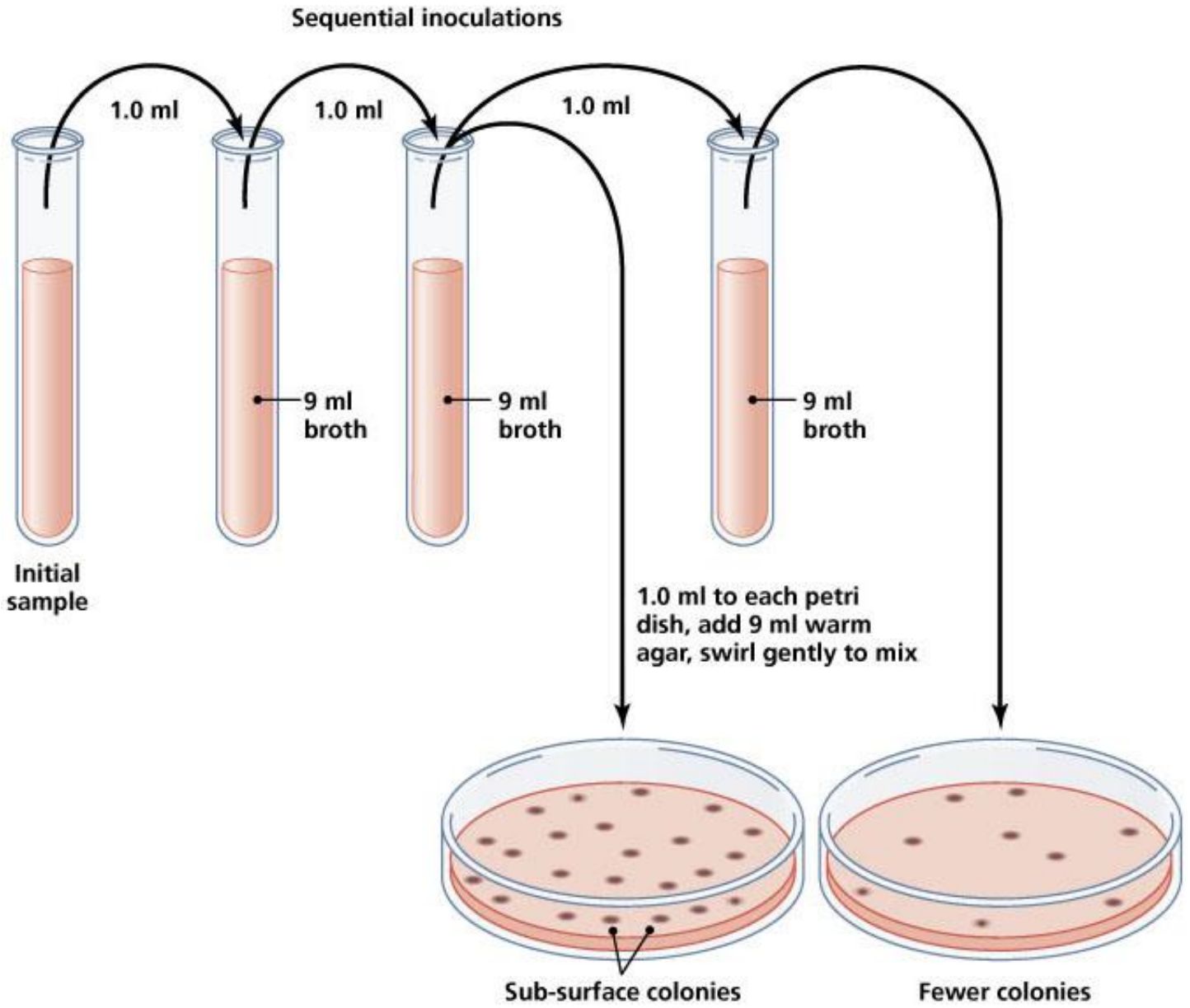



Figure 6.9

# Pour Plate Method

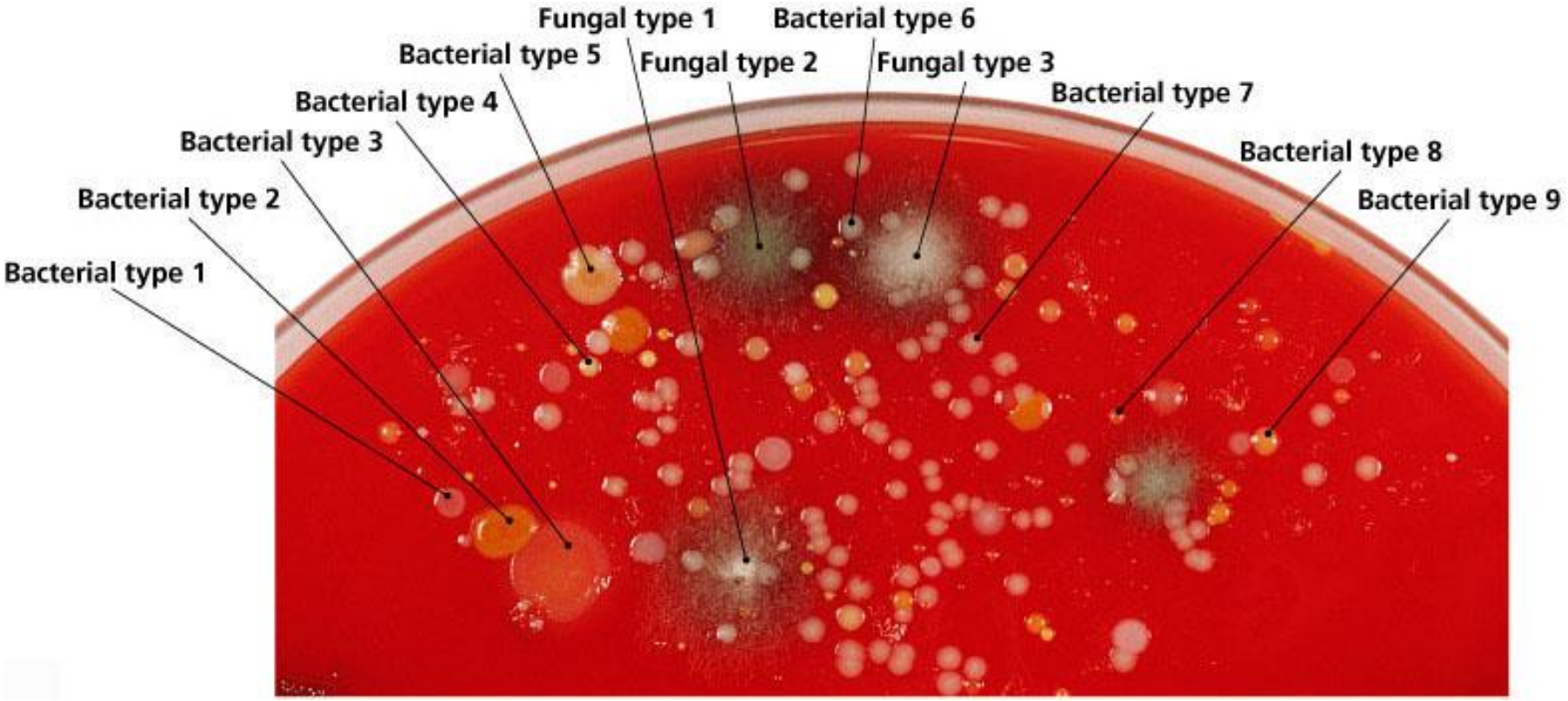



Figure 6.9

# Clinical Sampling

**Table 6.3 Clinical Specimens and the Methods Used to Collect Them**

| Type or Location of Specimen | Collection Method |
|---|---|
| Skin, accessible mucous membrane (including eye, outer ear, nose, throat, vagina, cervix, urethra) or open wounds | Sterile swab brushed across the surface; care should be taken not to contact neighboring tissues |
| Blood | Needle aspiration from vein, anticoagulants are included in the specimen transfer tube |
| Cerebrospinal fluid | Needle aspiration from subarachnoid space of spinal column |
| Stomach | Intubation, which involves inserting a tube into the stomach, often via a nostril |
| Urine | In aseptic collection, a catheter is inserted into the bladder through the urethra; in the "clean catch" method, initial urination washes the urethra, and the specimen is midstream urine |
| Lungs | Collection of sputum either dislodged by coughing or acquired via a catheter |
| Diseased tissue | Surgical removal (biopsy) |

Table 6.3

# Culture Media

- **Chemically <u>Defined Media</u>**: Exact chemical composition is known
- **Complex Media**: Exact chemical composition of some part are known while others are unknown (extracts of yeasts, meat, or plants)
  - Nutrient broth
  - Nutrient agar

# Culture Media

**TABLE 6.2** **A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *E. coli***

| Constituent | Amount |
|---|---|
| Glucose | 5.0 g |
| Ammonium phosphate, monobasic ($NH_4H_2PO_4$) | 1.0 g |
| Sodium chloride (NaCl) | 5.0 g |
| Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) | 0.2 g |
| Potassium phosphate, dibasic ($K_2HPO_4$) | 1.0 g |
| Water | 1 liter |

**TABLE 6.4** **Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria**

| Constituent | Amount |
|---|---|
| Peptone (partially digested protein) | 5.0 g |
| Beef extract | 3.0 g |
| Sodium chloride | 8.0 g |
| Agar | 15.0 g |
| Water | 1 liter |



Table 6.2 & 6.4

# Agar

- Complex polysaccharide
- Used as solidifying agent
  - in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C

What was used before agar?

# Culture Media

- Types of general culture media
  - Defined media
  - Complex media
  - Selective media
  - Differential media
  - Enriched Media
  - Anaerobic media

# Selective Media

- Selects _____ the growth of some microbes while selecting for the growth of other microbes.

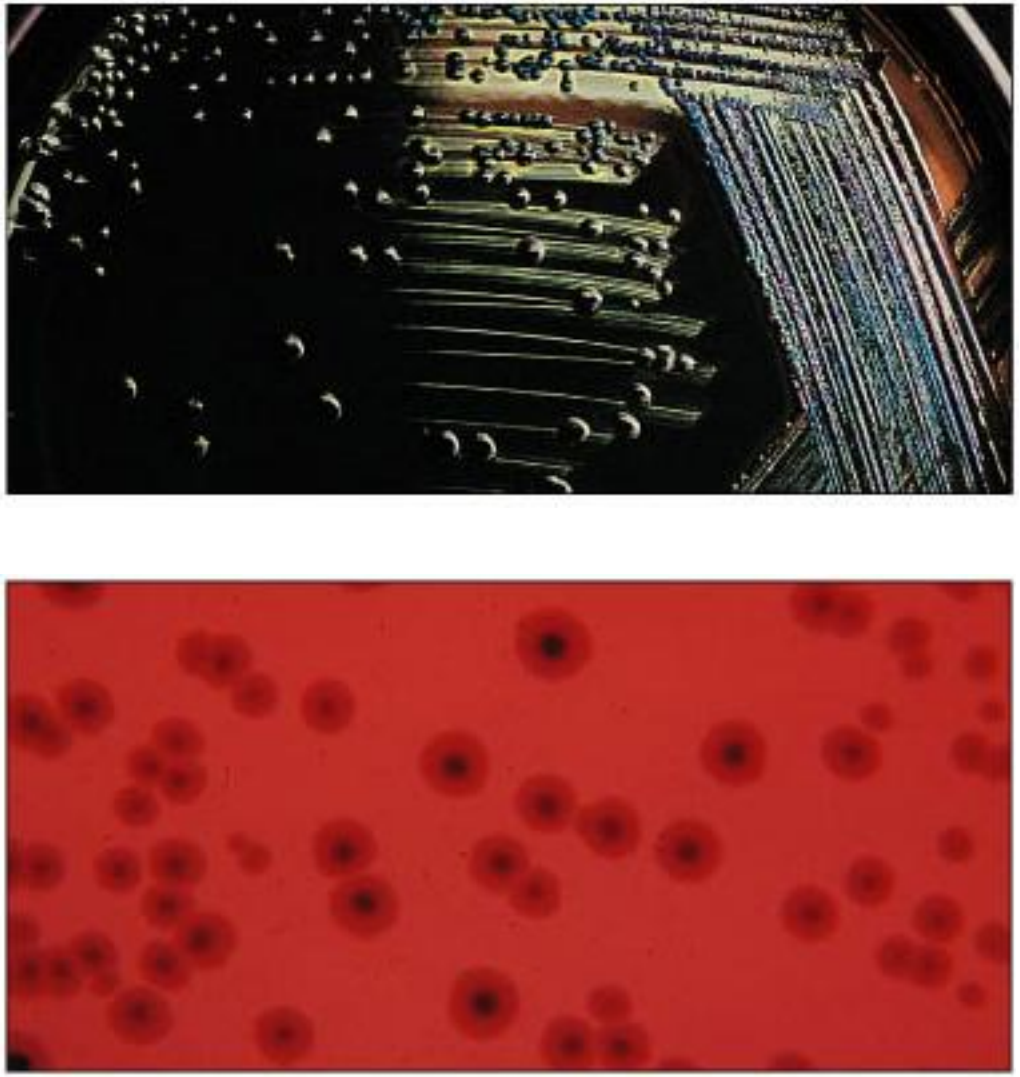




Figure 6.9b, c

# Differential Media

- Make it easy to distinguish colonies of different microbes.
  Allows for differentiation between different types of microbes (usually based on color)



Figure 6.9a

# Enrichment Media

- Encourages growth of desired microbe
- These are usually fastidious bacteria

Chocolate agar

# Anaerobic Culture Methods

- Anaerobic jar

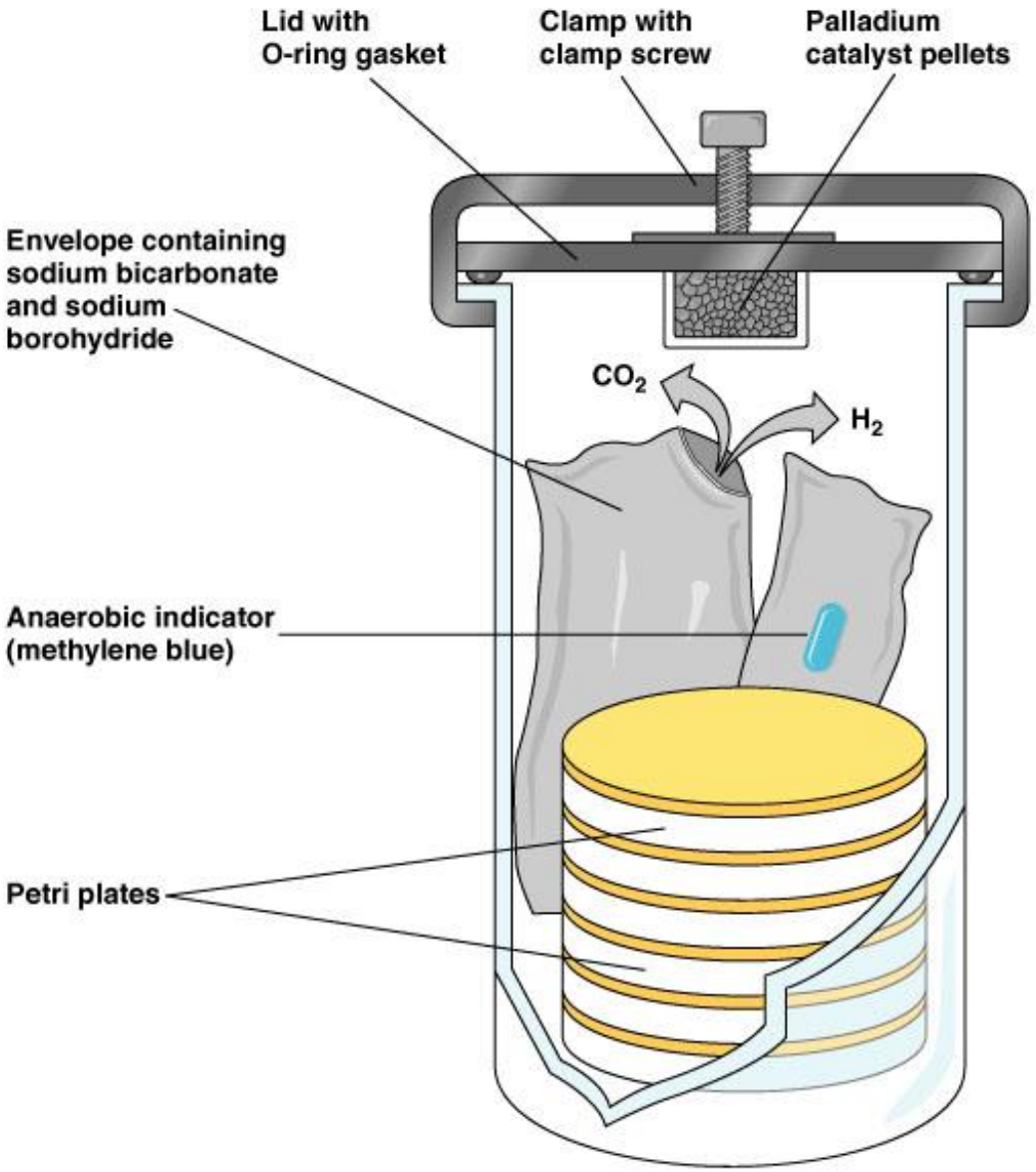



Figure 6.5

# Anaerobic Culture Methods

- Reducing media
  - Contain chemicals (thioglycollate or oxyrase) that combine free $O_2$
  - Heated to drive off $O_2$

# Anaerobic Culture Methods

- Anaerobic chamber

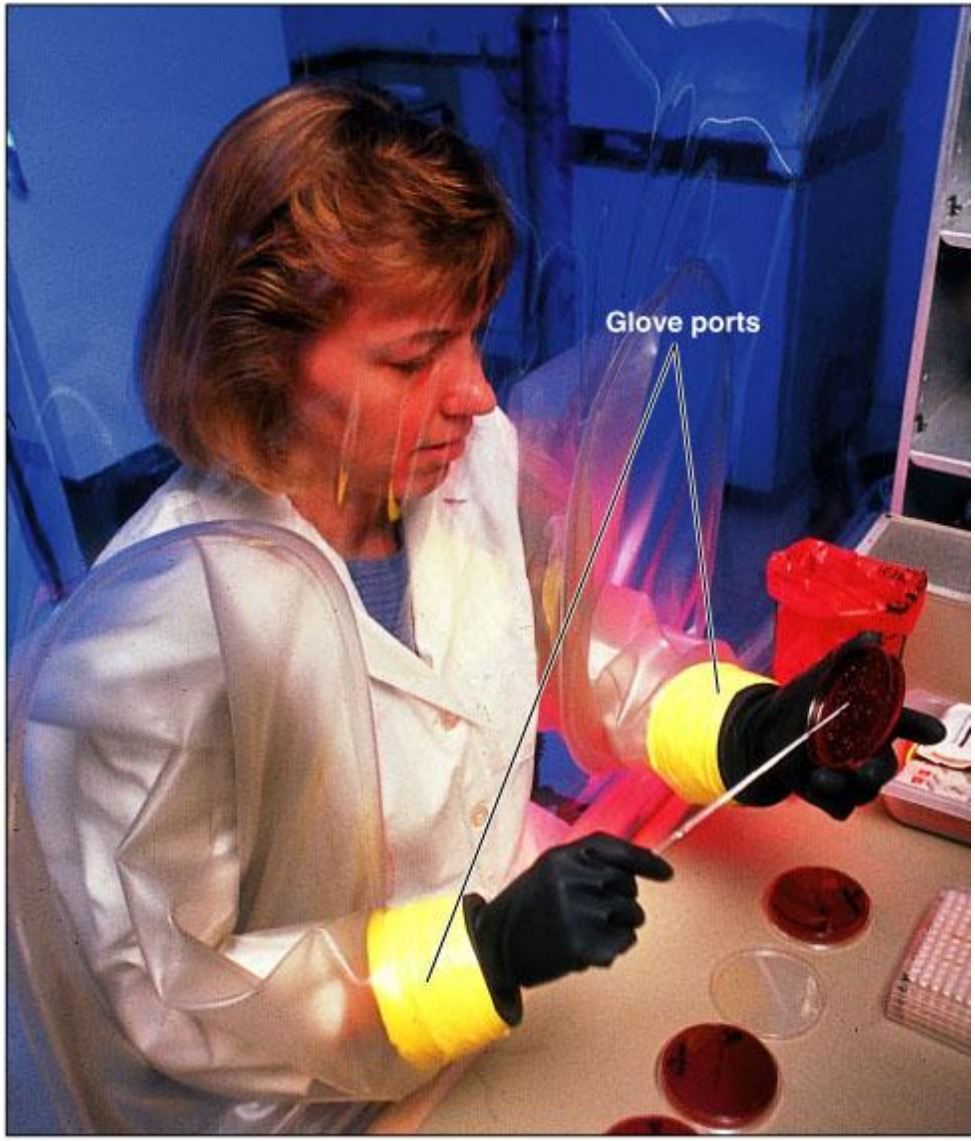
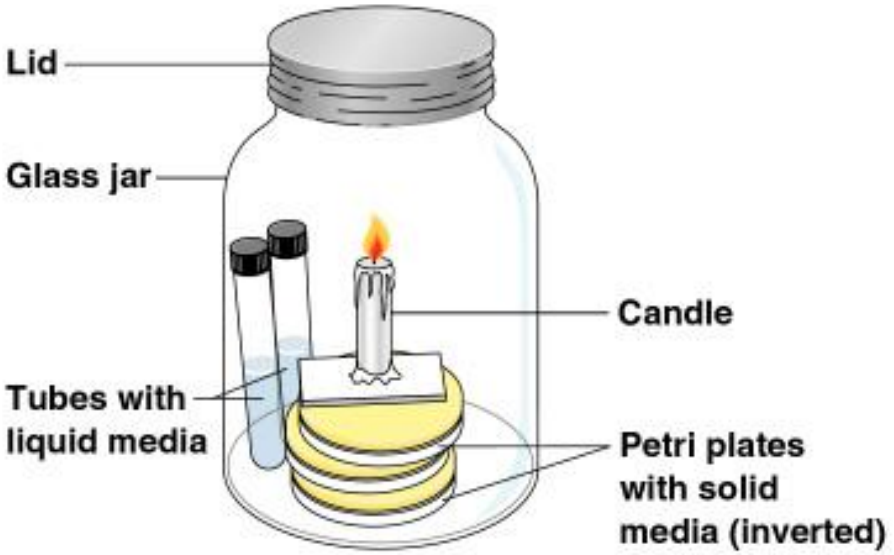



Figure 6.6

# Capnophiles require high $CO_2$

- Candle jar




- $CO_2$-packet

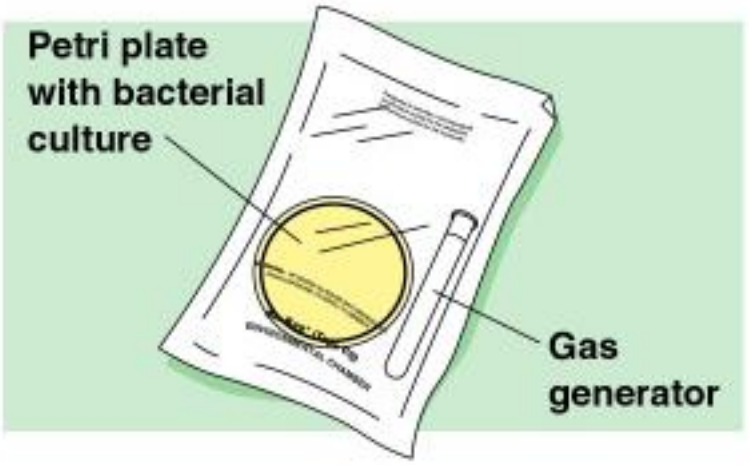





Figure 6.7

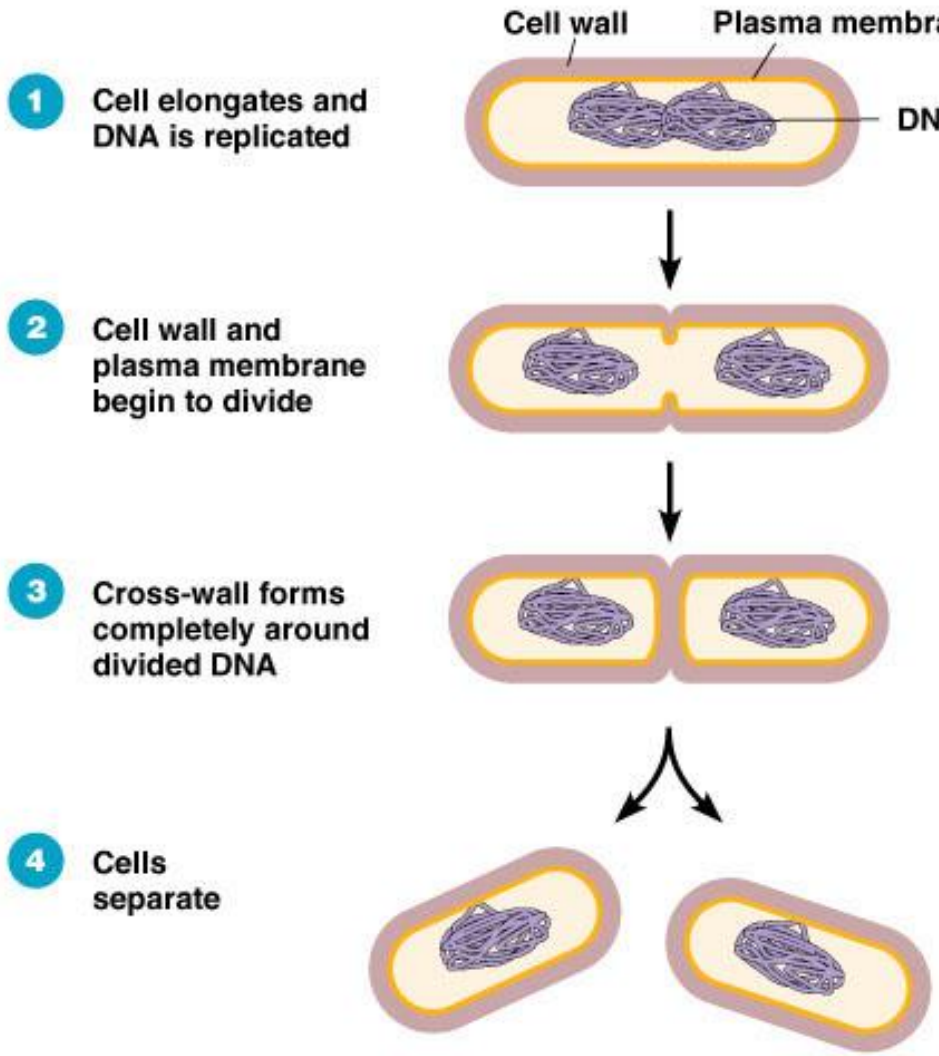
# Preserving Cultures

- Refrigeration:
- Freezing:
- Deep-freezing:  -70°to -95°C
- Lyophilization (freeze-drying): Frozen (-54° to -72°C) and dehydrated in a vacuum

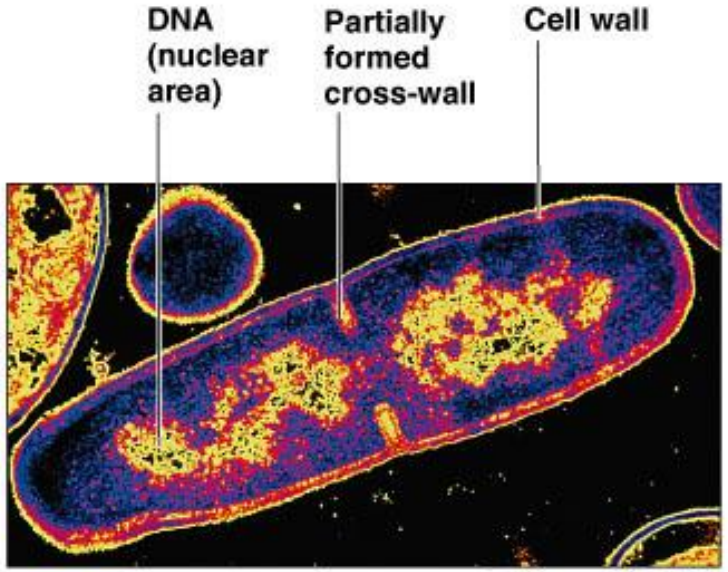
# Reproduction of Microbes

- Prokaryotes
  - Binary fission
- Eukaryotes
  - Sexual and Asexual (mitosis, meiosis, budding, conidiospores, fragmentation of filaments, etc.)

# Binary Fission




**(a)** A diagram of the sequence of cell division.

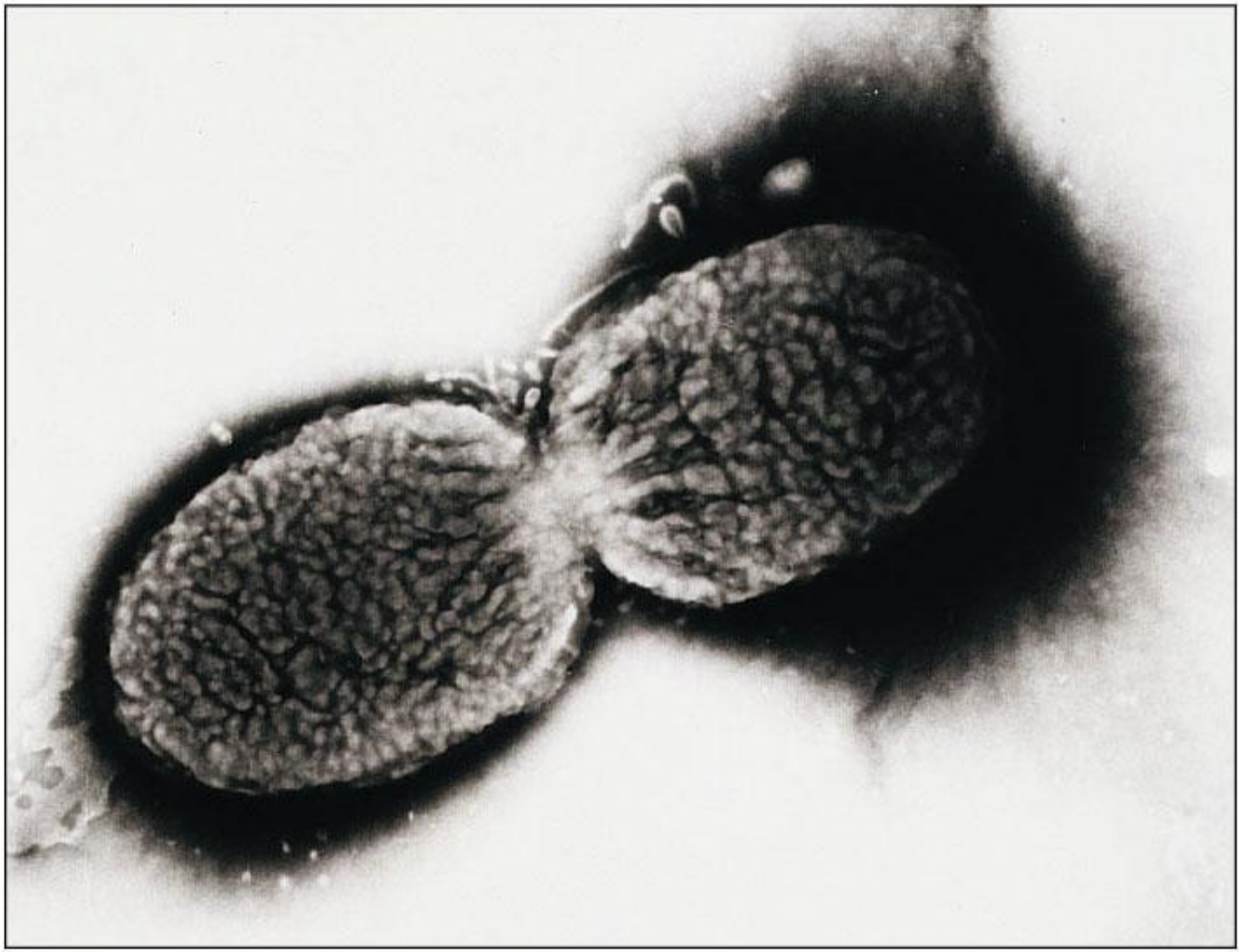



**(b)** A thin section of a cell of *Bacillus licheniformis* starting to divide.



Figure 6.11

# Growth of Microbial Populations



(b)

Figure 6.17

# Growth of Microbial Populations

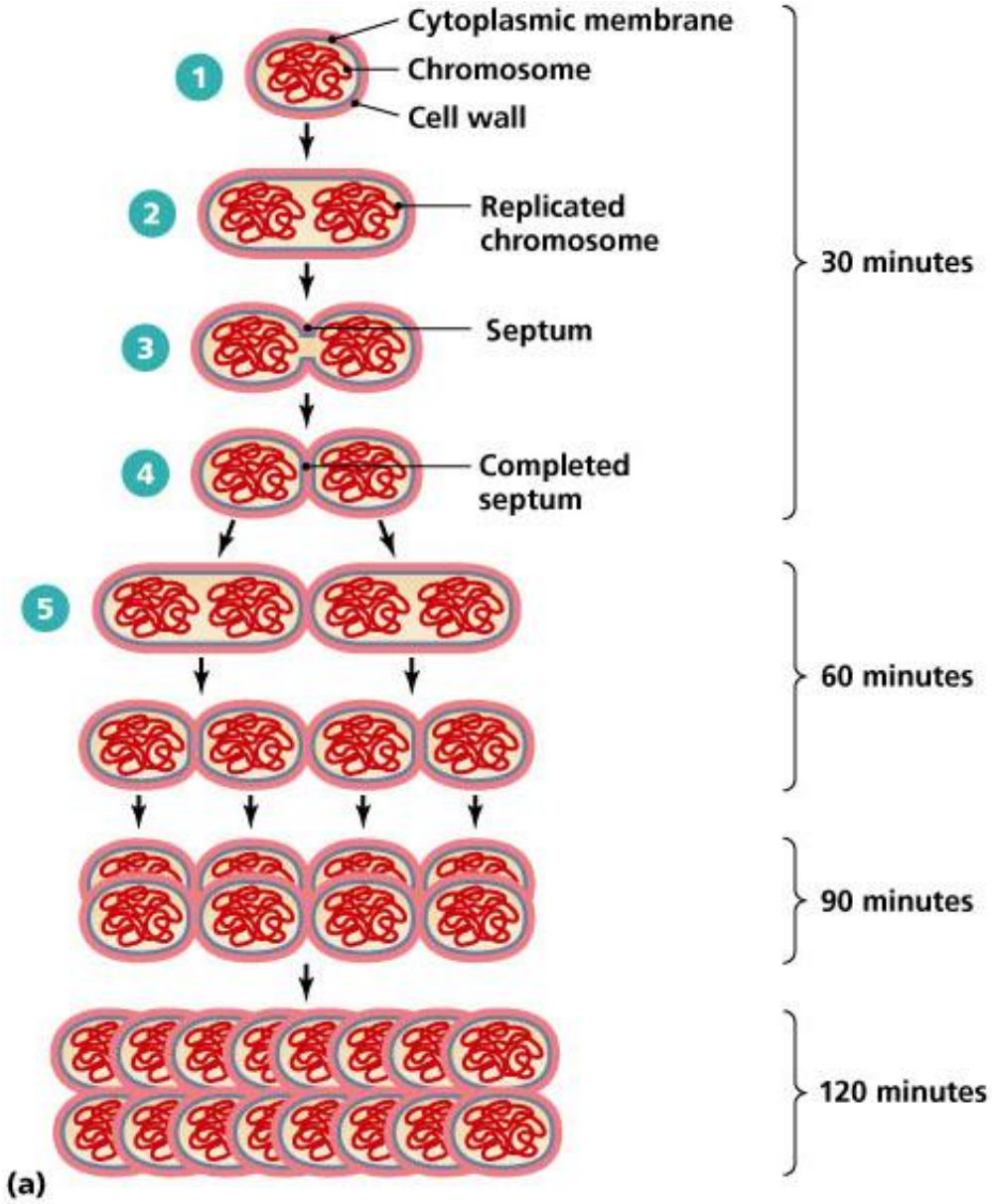



Figure 6.17

# Arithmetic Versus Logarithmic Growth

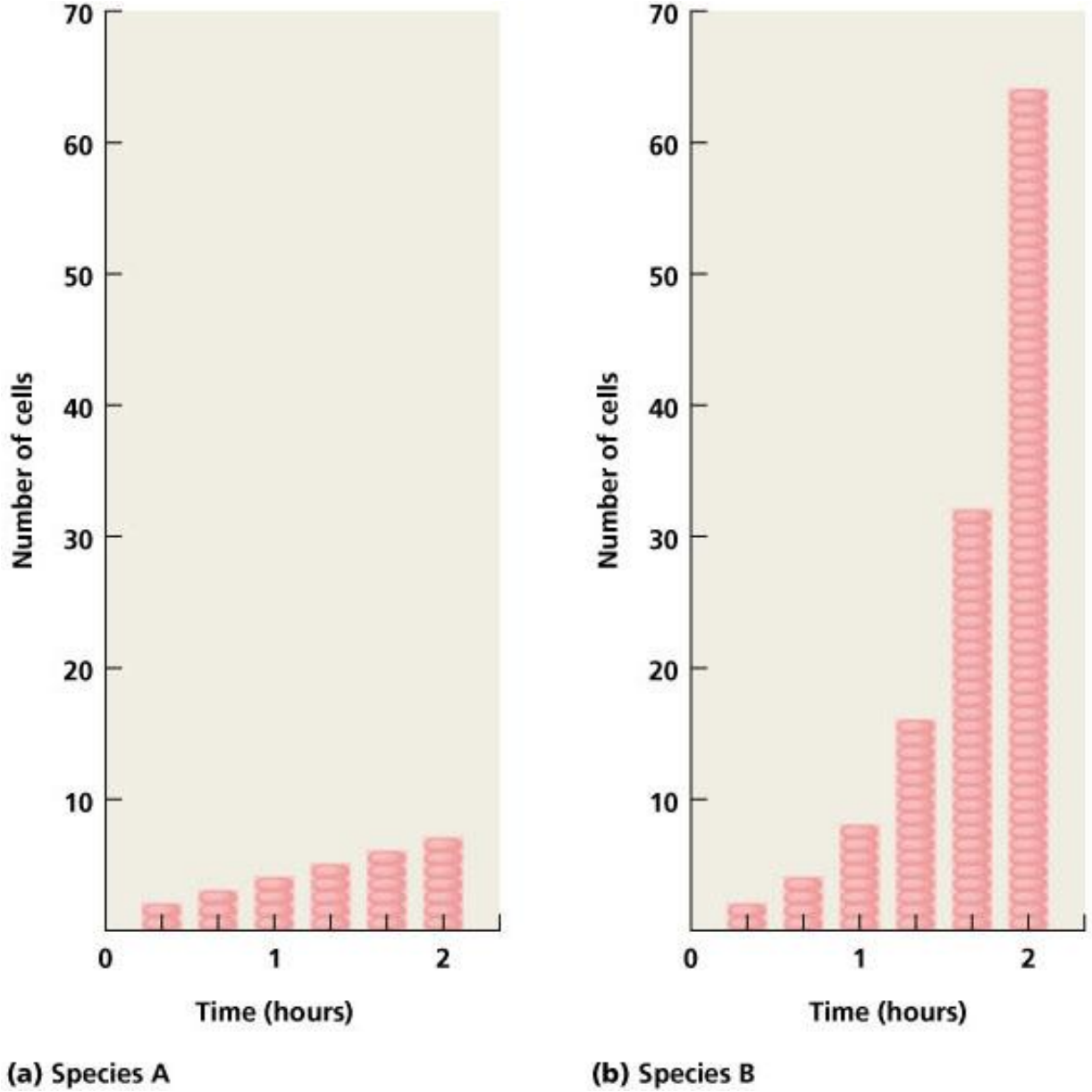



Figure 6.18

| Generation Number | Number of Cells | $Log_{10}$ of Number of Cells |
|---|---|---|
| 0 | 1 | 0 |
| 5 ($2^5$) = | 32 | 1.51 |
| 10 ($2^{10}$) = | 1,024 | 3.01 |
| 15 ($2^{15}$) = | 32,768 | 4.52 |
| 16 ($2^{16}$) = | 65,536 | 4.82 |
| 17 ($2^{17}$) = | 131,072 | 5.12 |
| 18 ($2^{18}$) = | 262,144 | 5.42 |
| 19 ($2^{19}$) = | 524,288 | 5.72 |
| 20 ($2^{20}$) = | 1,048,576 | 6.02 |

**(b)**

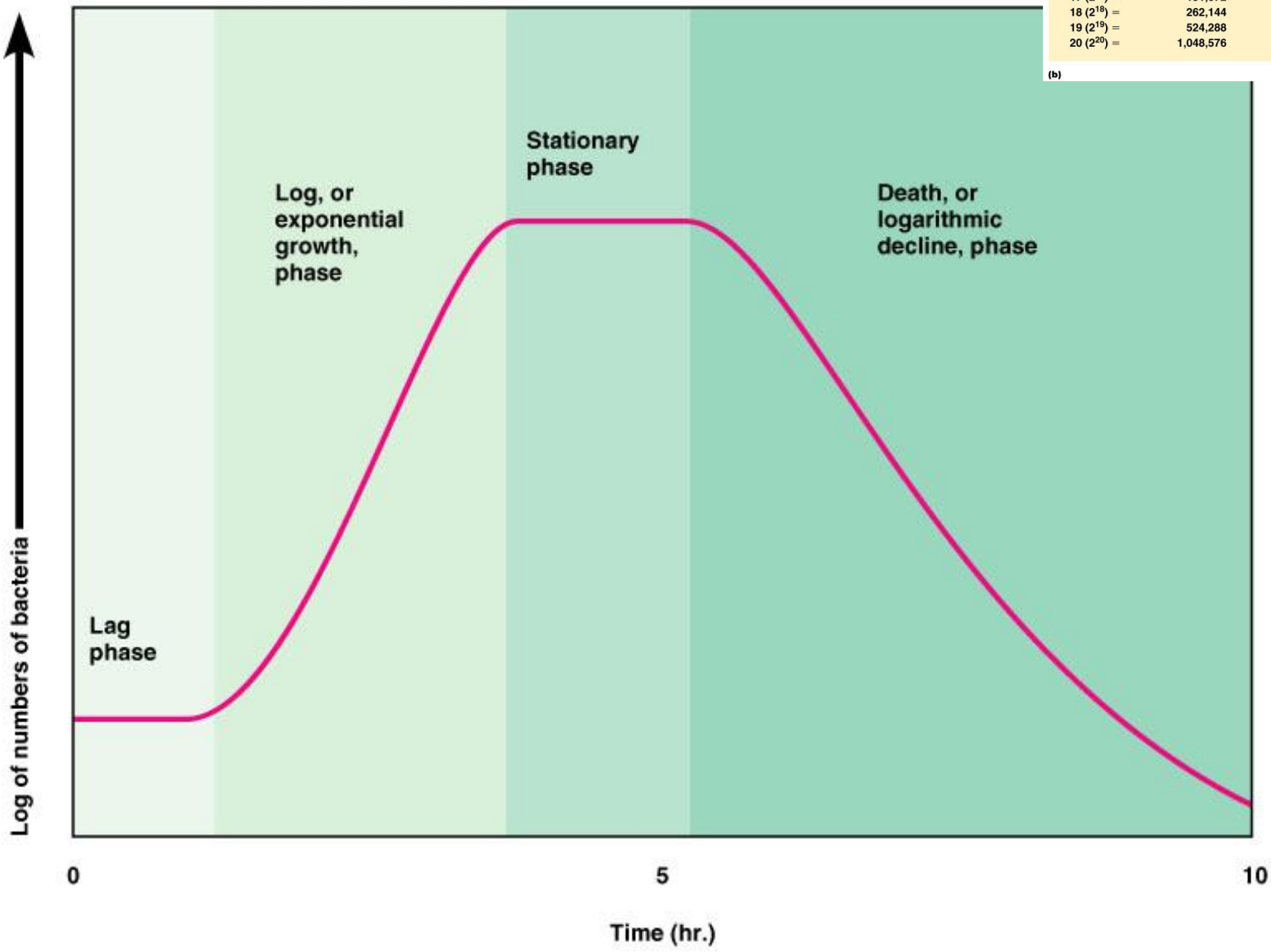


Figure 6.12b

# Phases of Microbial Growth

| Generation Number | Number of Cells | $Log_{10}$ of Number of Cells |
|---|---|---|
| 0 | 1 | 0 |
| 5 ($2^5$) = | 32 | 1.51 |
| 10 ($2^{10}$) = | 1,024 | 3.01 |
| 15 ($2^{15}$) = | 32,768 | 4.52 |
| 16 ($2^{16}$) = | 65,536 | 4.82 |
| 17 ($2^{17}$) = | 131,072 | 5.12 |
| 18 ($2^{18}$) = | 262,144 | 5.42 |
| 19 ($2^{19}$) = | 524,288 | 5.72 |
| 20 ($2^{20}$) = | 1,048,576 | 6.02 |

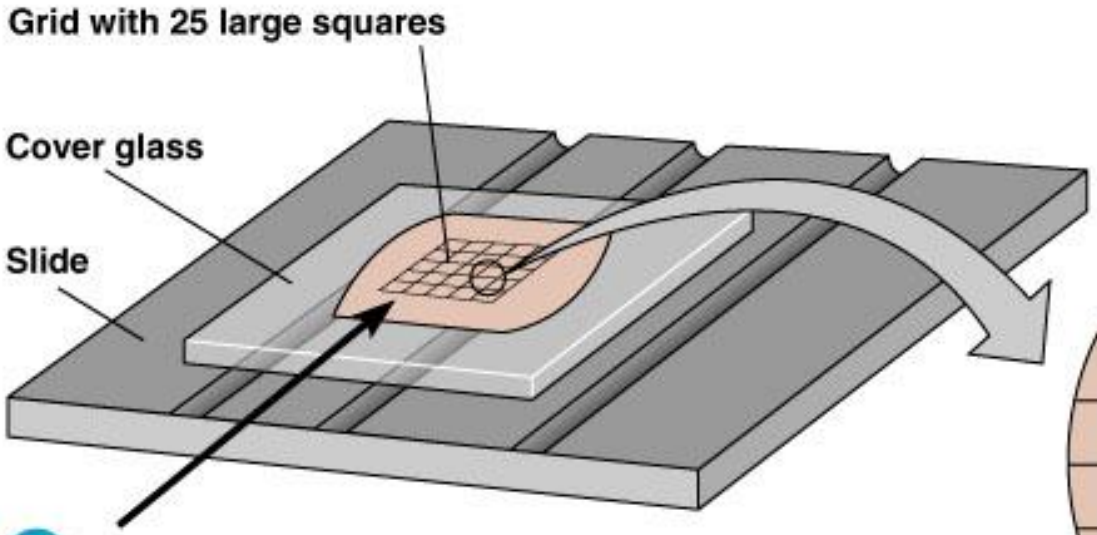
(b)



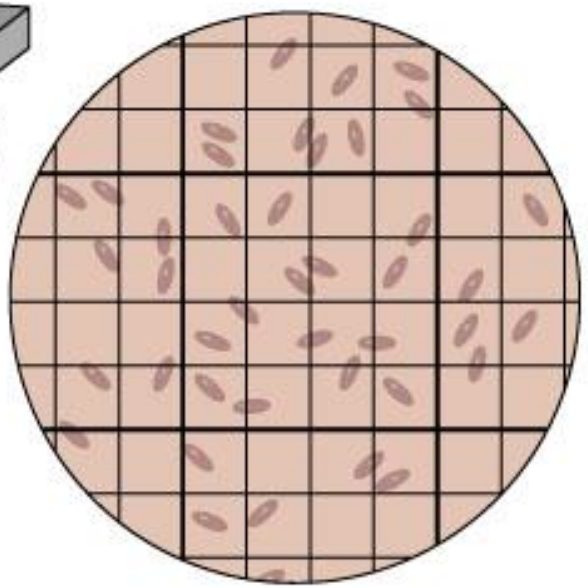
# Measuring Microbial Growth

- Direct Methods
  - Microscopic counts
  - (Viable) Plate Counts
  - Membrane _____
  - Electronic Counters
  - Most Probable Number

# Direct Measurements of Microbial Growth




1 Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.



## Direct Microscope Count

$$\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}$$

$$\frac{14}{8 \times 10^{-7}} = 17{,}500{,}000$$

3 Microscopic count: All cells in several large squares are counted, and the numbers are averaged. The large square shown here has 14 bacterial cells.



Figure 6.19

# Direct Measurements of Microbial Growth

- Plate Counts: Perform serial dilutions of a sample

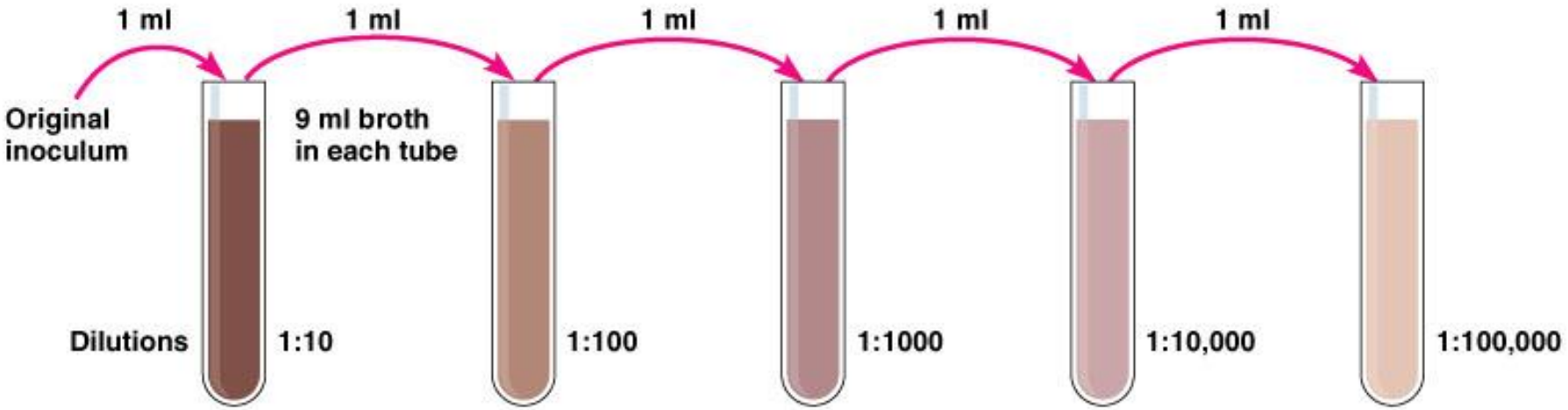





Figure 6.15, top portion

# Plate Count

- Inoculate Petri plates from serial dilutions

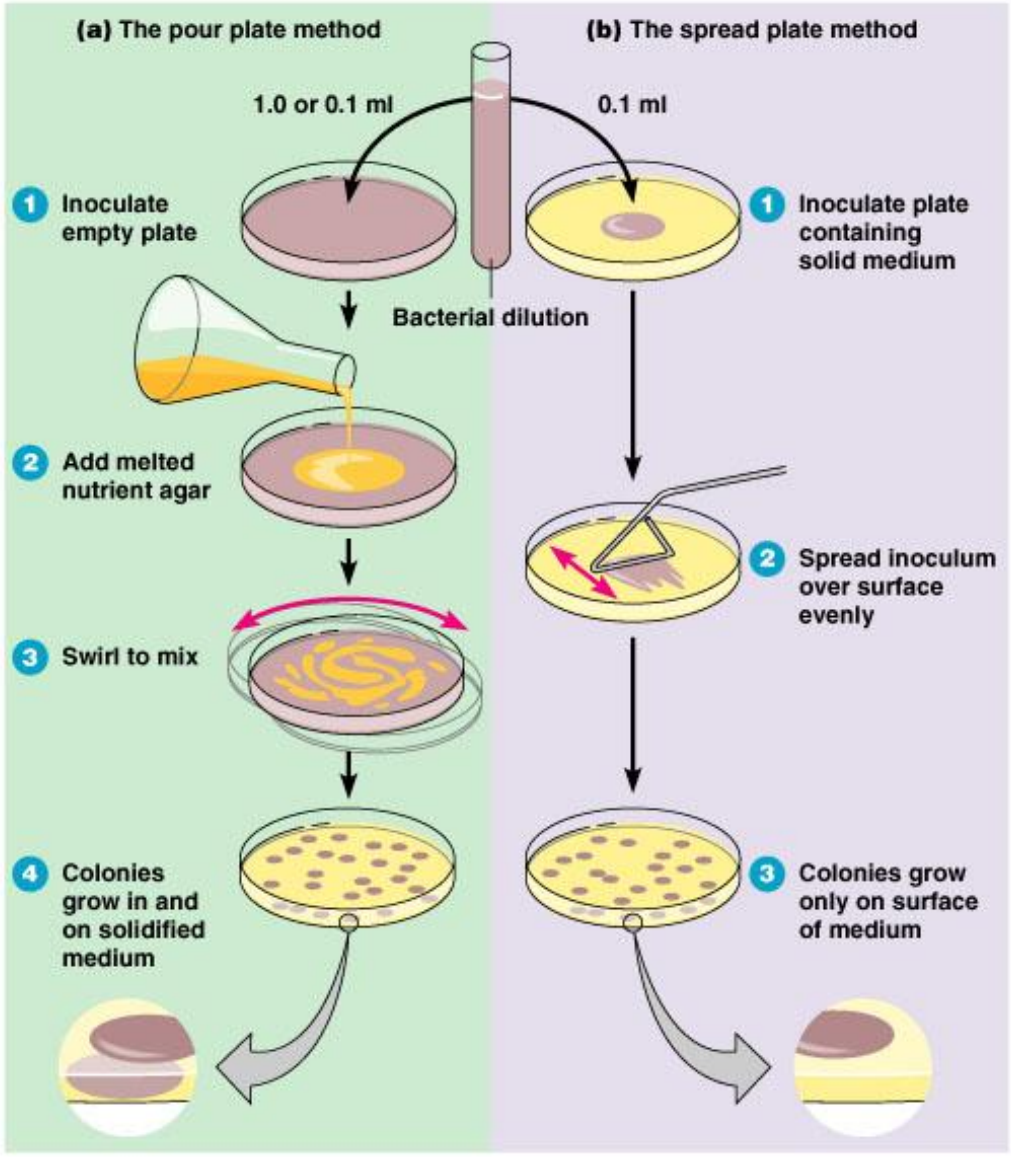





Figure 6.16

# Plate Count

- After incubation, count colonies on plates that have 25-250 colonies (CFUs)

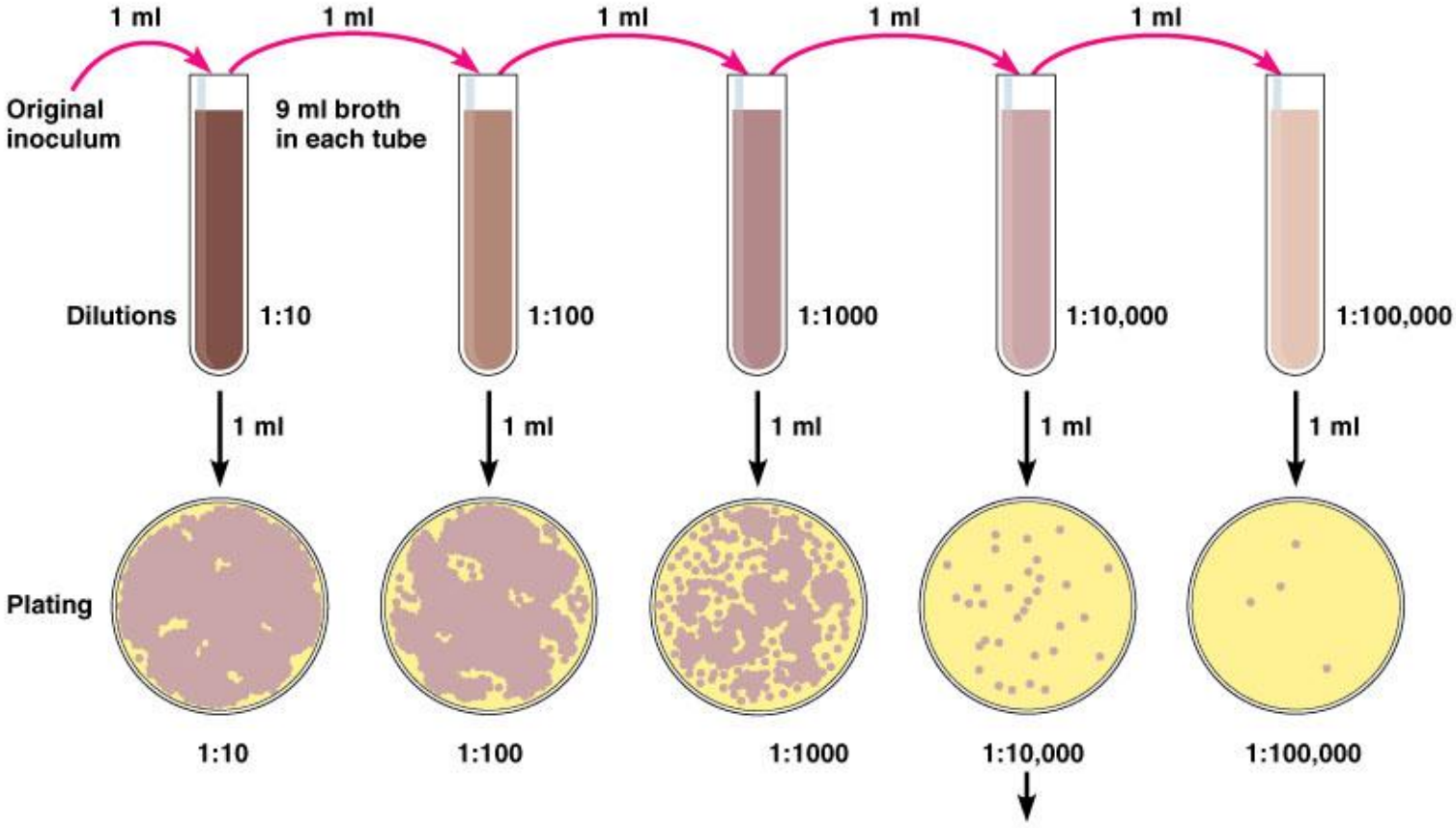





Figure 6.15

# Membrane Filtration

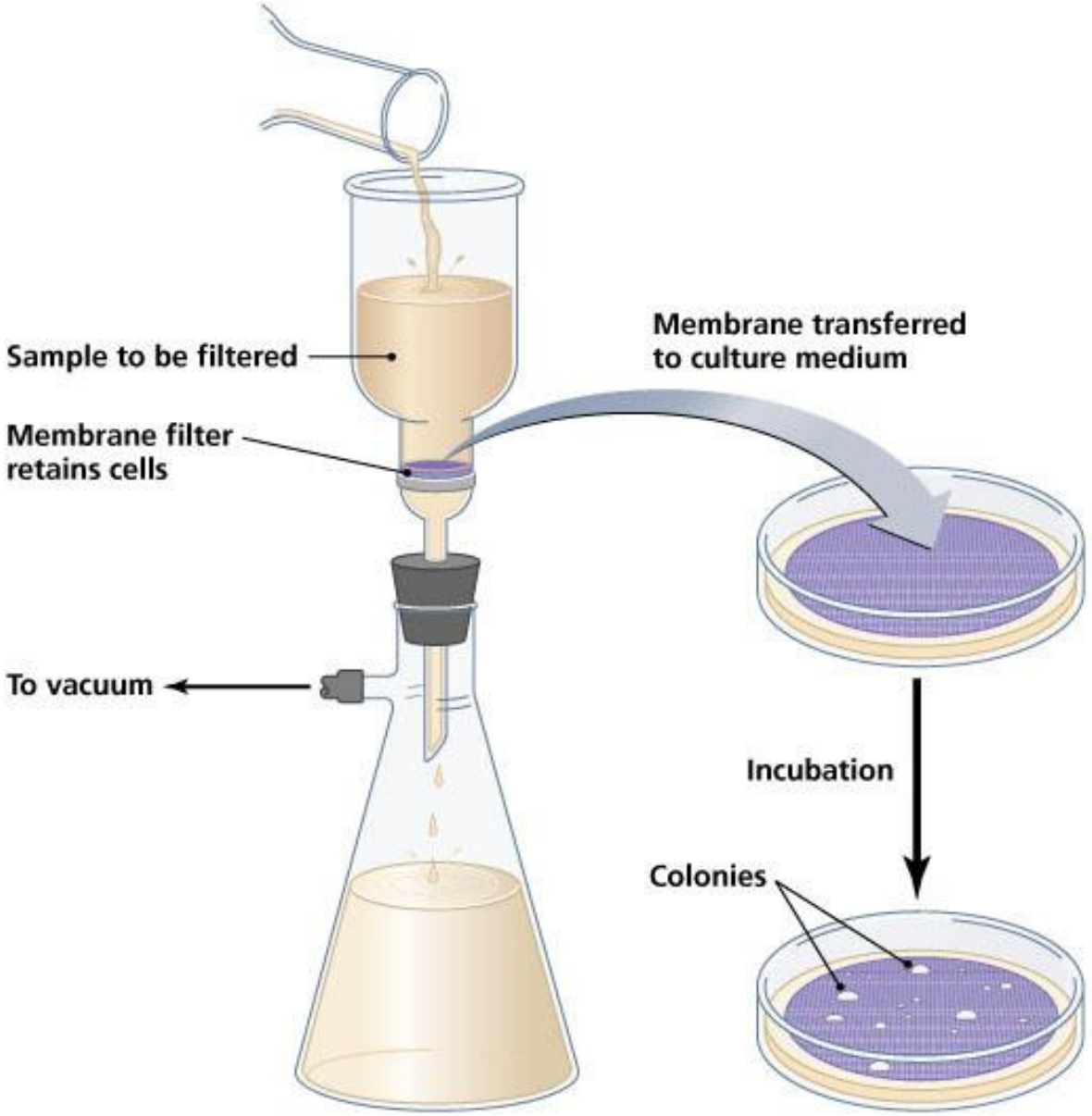
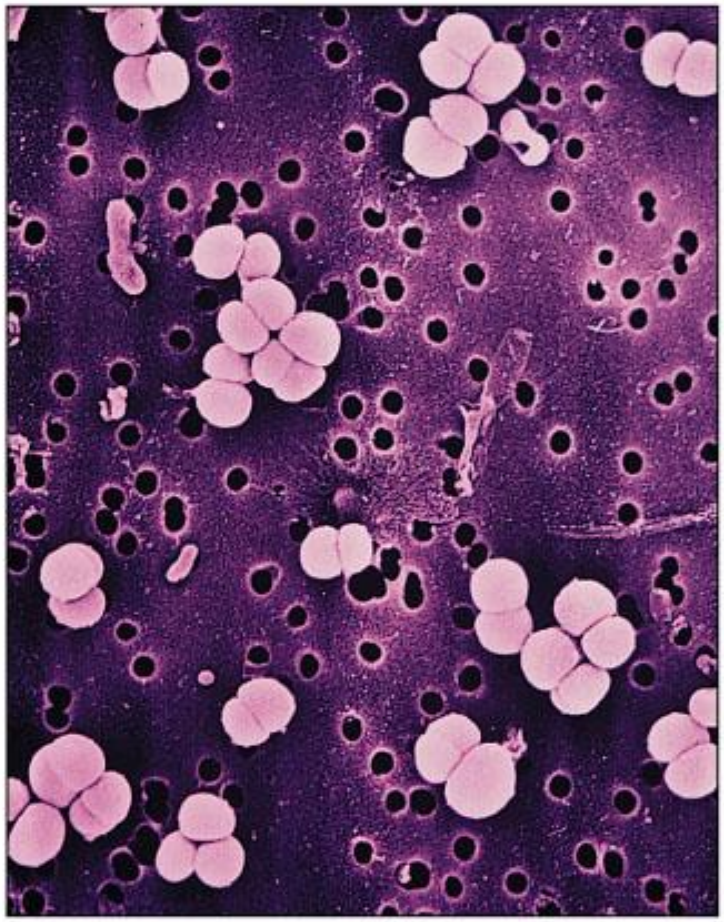



Figure 6.22a

# Direct Measurements of Microbial Growth

- Filtration



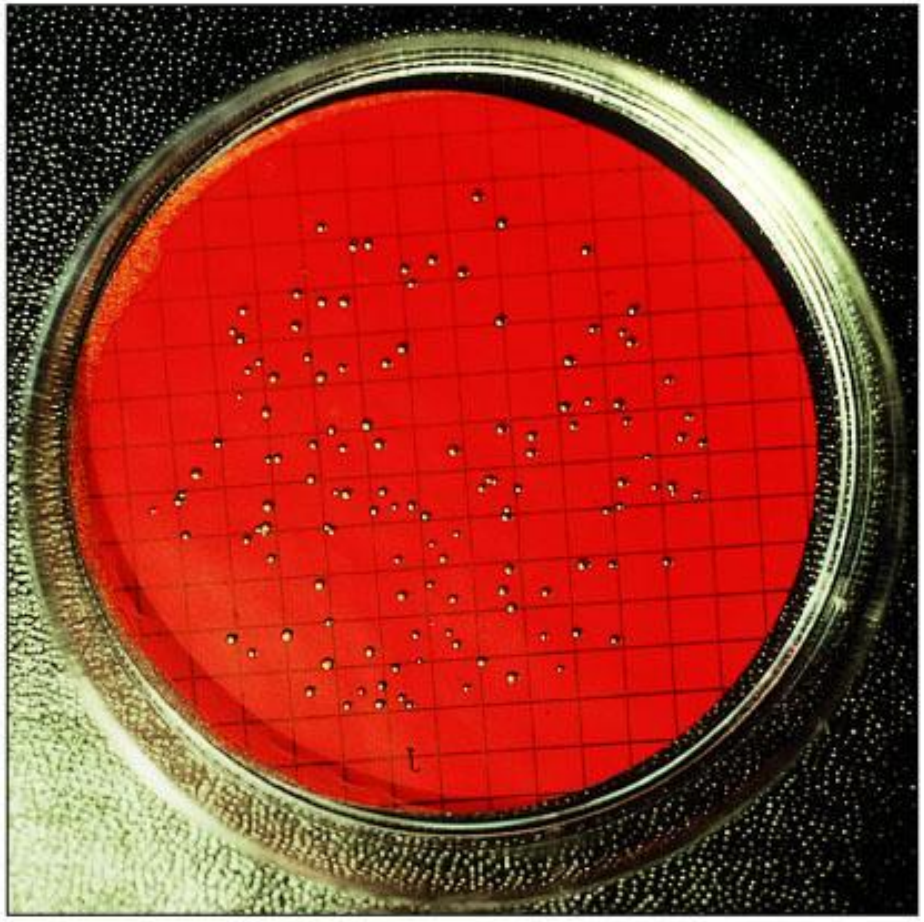




Figure 6.17a, b

# Most Probable Number

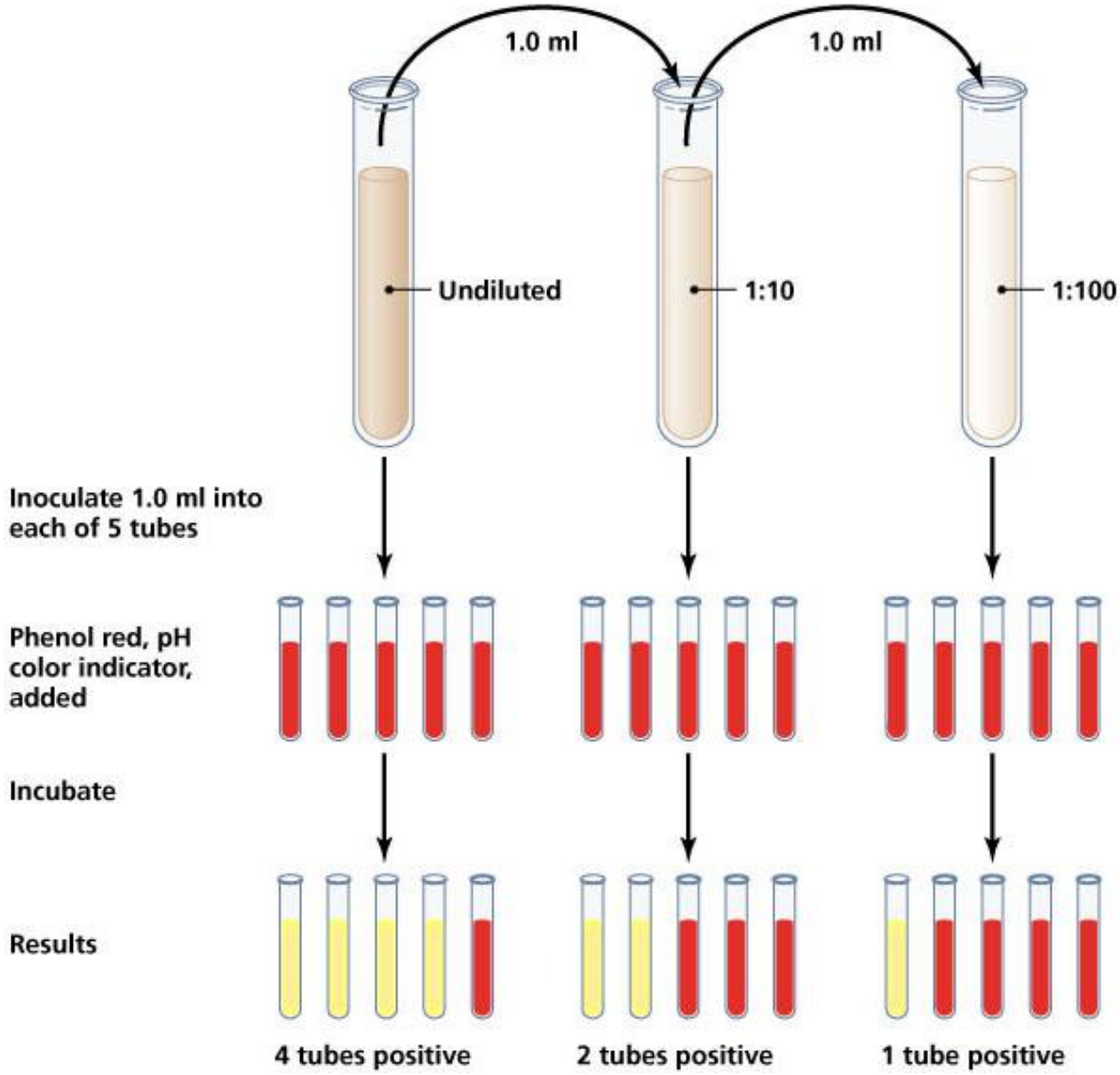



Figure 6.24

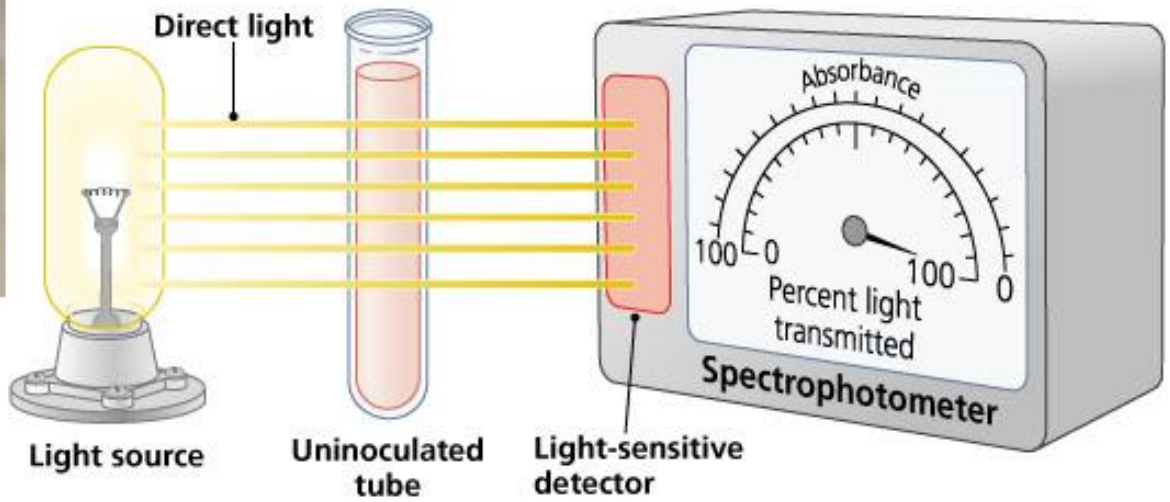
# Measuring Microbial Growth

- Indirect Methods
  - Metabolic Activity
  - Dry Weight
  - Turbidity

# Turbidity and Spectrophotometric Measurement

Figure 6.25a

# Turbidity and Spectrophotometric Measurement



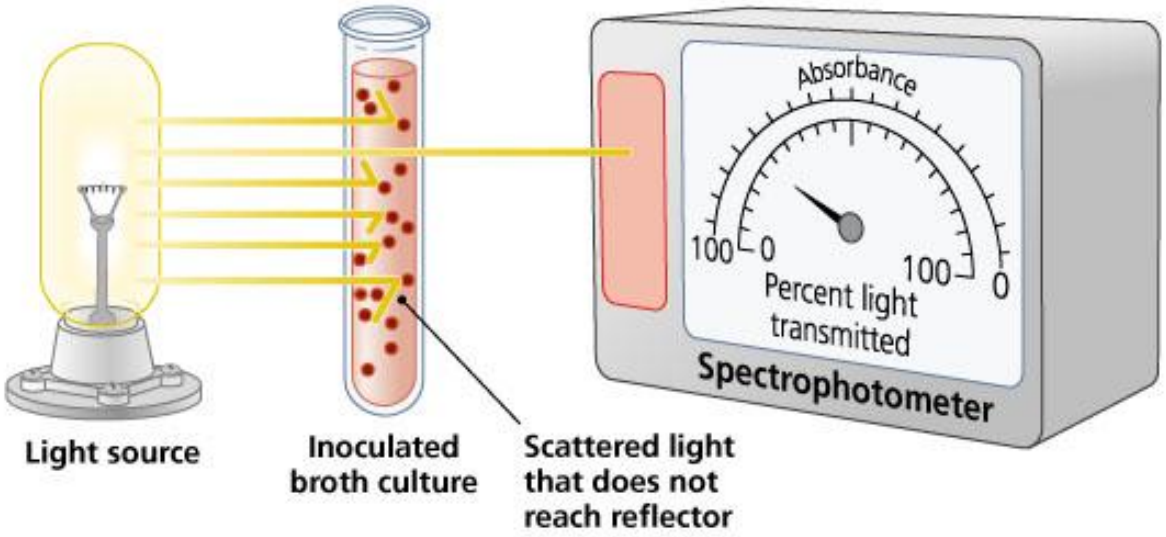






Figure 6.25c