Chapter 6
Microbial Growth

Microbial Growth
- Increase in number of cells, not cell size
- Populations
- Colonies

The Requirements for Growth
- Physical requirements
- Temperature
- pH
- Osmotic pressure
- Chemical requirements
- Carbon
- Nitrogen, sulfur, and phosphorous
- Trace elements
- Oxygen
- Organic growth factor

Physical Requirements
- Temperature
  - Maximum
  - Optimum
  - Minimum

- Psychrotrophs
  - Grow between 0°C and 20–30°C
  - Cause food spoilage

pH
- Most bacteria grow between pH 6.5 and 7.5
- Molds and yeasts grow between pH 5 and 6
- Acidophiles grow in acidic environments

Osmotic Pressure
- Hypertonic environments, or an increase in salt or sugar, cause plasmolysis
- Extreme or obligate halophiles require high osmotic pressure
- Facultative halophiles tolerate high osmotic pressure

Chemical Requirements

Carbon
- Structural organic molecules, energy source
- Chemoheterotrophs use organic carbon sources
- Autotrophs use CO_2

Nitrogen
- In amino acids and proteins
- Most bacteria decompose proteins
- Some bacteria use NH_4^+ or NO_3^-
- A few bacteria use N_2 in nitrogen fixation

Sulfur
- In amino acids, thiamine, and biotin
- Most bacteria decompose proteins
- Some bacteria use SO_4^{2-} or H_2S

Phosphorus
- In DNA, RNA, ATP, and membranes
- PO_4^{3-} is a source of phosphorus

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

Toxic Oxygen
- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_2^-
- Peroxide anion: O_2^{2-}
- Hydroxyl radical (OH•)

Organic Growth Factors
- Organic compounds obtained from the environment
- Vitamins, amino acids, purines, and pyrimidines

Biofilms
- Microbial communities
- Form slime or hydrogels
- Bacteria attracted by chemicals via quorum sensing
- Share nutrients
- Sheltered from harmful factors

- Patients with indwelling catheters received contaminated heparin
  - Bacterial numbers in contaminated heparin were too low to cause infection
  - 84–421 days after exposure, patients developed infections
  - Pseudomonas fluorescens was cultured from the catheters
- What happened?

Culture Media
- Culture medium: Nutrients prepared for microbial growth
- Sterile: No living microbes
- Inoculum: Introduction of microbes into medium
- Culture: Microbes growing in/on culture medium

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

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Culture Media
- Chemically defined media: Exact chemical composition is known
- Complex media: Extracts and digests of yeasts, meat, or plants
- Nutrient broth
- Nutrient agar

Anaerobic Culture Methods
- Reducing media
- Contain chemicals (thioglycolate or oxyrase) that combine O_2
- Heated to drive off O_2

Capnophiles
- Microbes that require high CO2 conditions
- CO2 packet

Candle jar

Biosafety Levels
- 1: No special precautions
- 2: Lab coat, gloves, eye protection
- 3: Biosafety cabinets to prevent airborne transmission
- 4: Sealed, negative pressure
- Exhaust air is filtered twice

Selective Media
- Suppress unwanted microbes and encourage desired microbes

Differential Media
- Make it easy to distinguish colonies of different microbes.

Enrichment Culture
- Encourages growth of desired microbe
- Assume a soil sample contains a few phenol-degrading bacteria and thousands of other bacteria
- Inoculate phenol-containing culture medium with the soil, and incubate
- Transfer 1 ml to another flask of the phenol medium, and incubate
- Transfer 1 ml to another flask of the phenol medium, and incubate
- Only phenol-metabolizing bacteria will be growing

Obtaining Pure Cultures
- A pure culture contains only one species or strain
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a colony-forming unit (CFU)
- The streak plate method is used to isolate pure cultures

The Streak Plate Method

Preserving Bacterial Cultures
- Deep-freezing: –50° to –95°C
- Lyophilization (freeze-drying): Frozen (–54° to –72°C) and dehydrated in a vacuum

Reproduction in Prokaryotes
- Binary fission
- Budding
- Conidiospores (actinomycetes)
- Fragmentation of filaments

Measuring Microbial Growth
- Generation Time
- Bacterial Growth Curve
- Phases of Growth

Measuring Microbial Growth

Serial Dilutions

Direct Methods
- Plate counts
- Filtration
- MPN
- Direct microscopic count

Indirect Methods
- Turbidity
- Metabolic activity
- Dry weight