Chapter 4 Dynamics of Prokaryotic Growth

Summary Outline

4.1 Obtaining a **pure culture**

A. About one-tenth of one percent of bacteria can be cultured in the laboratory.
B. Cultivating bacteria on a solid medium
    1. A single bacterial cell will multiply to form a visible colony.
    2. **Agar** is used to solidify nutrient-containing broth.
C. The **streak plate method** is used to isolate bacteria in order to obtain a pure culture.
D. Maintaining stock cultures
    1. **Stock cultures** can be used as an **inoculum** in later experiments.
    2. **Stock cultures** can be **stored on an agar slant in the refrigerator**, **frozen in a glycerol solution** or **lyophilized**.

4.2 Principles of bacterial growth

A. Most bacteria multiply by **binary fission**.
B. **Microbial growth** is an **increase in the number of cells** in a population.
C. The time required for a population to double in number is the **generation time**.

4.3 **Environmental factors** that influence microbial growth

A. **Temperature requirements**
    1. **Psychrophiles** have an optimum between -5°C and 15°C.
    2. **Psychrotrophs** have an optimum between 20°C and 30°C
    3. **Mesophiles** have an optimum between 25°C and 45°C.
    4. **Thermophiles** have an optimum between 45°C and 70°C.
    5. **Hyperthermophiles** have an optimum between 70°C and 110°C.
    6. Storage of foods at refrigeration temperatures retards spoilage because it limits the growth of mesophiles.
    7. Some microorganisms can inhabit certain parts of the body but not others because of temperature differences.
B. **Oxygen requirements**
    1. **Obligate anaerobes** cannot multiply if oxygen is present.
    2. **Facultative anaerobes** can multiply if oxygen is present but can also grow without it.
    3. **Microaerophiles** require small amounts of oxygen but higher concentrations are inhibitory.
    4. **Aerotolerant anaerobes** are indifferent to oxygen.
    5. Oxygen can be converted to **superoxide** and **hydrogen peroxide**, both of which are toxic. **Superoxide dismutase** and **catalase** can break these down.
C. **PH**
    1. Most bacteria live within the pH range of 5 to 8.
    2. **Acidophiles** grow optimally at a pH below 5.5.
    3. **Alkaliphiles** grow optimally at a pH above 8.5.
D. **Water** availability
    1. All microorganisms require water for growth.
    2. If the solute concentration is higher in the medium than in the cell, water diffuses out of the cell, causing plasmolysis.
    3. **Halophiles** have adapted to live in high salt environments.

4.4 **Nutritional factors** that influence microbial growth

A. Required elements

1. The major elements make up cell constituents and include **carbon, nitrogen, sulfur** and **phosphorus**.
2. **Heterotrophs** use organic carbon.
3. **Autotrophs** fix CO_2.
4. **Trace elements** are required in very minute amounts.

B. **Growth factors** are cell constituents such as amino acids and vitamins that the cell cannot synthesize.
1. Organisms derive energy either from sunlight or from the oxidation of chemical compounds.
2. Nutritional diversity

C. Prokaryotes use diverse sources of carbon and energy.
1. **Photoautotrophs** use the energy of sunlight and the carbon in the atmosphere to make organic compounds.
2. **Chemolithoautotrophs** use inorganic compounds for energy and derive their carbon from CO_2.
3. **Photoheterotrophs** use the energy of sunlight and derive their carbon from organic compounds.
4. **Chemoorganoheterotrophs** use organic compounds for energy and as a carbon source.

4.5 Cultivating prokaryotes in the laboratory

A. General categories of culture media
1. **Complex medium** contains a variety of ingredients such as peptones and extracts. (Examples: nutrient agar, blood agar and chocolate agar)
2. A **chemically defined medium** is composed of precise mixtures of pure chemicals; an example is glucose-salts medium.

B. Special types of culture media
1. A **selective medium** inhibits organisms other than the one being sought (Examples**: Thayer Martin agar** and **MacConkey agar**)
2. A **differential medium** contains a substance that certain bacteria change in a recognizable way (Examples: **Blood agar** and **MacConkey agar**)

C. Providing appropriate **atmospheric conditions**
1. A **candle jar** provides **increased CO_2**, which enhances the growth of many medically important bacteria.
2. **Microaerophilic bacteria** are incubated in a gas-tight jar along with atmospheric oxygen to form water.
3. **Anaerobes** may be cultivated in either an **anaerobe jar** or a medium that incorporates a reducing agent.
4. An enclosed chamber that maintains anaerobic conditions can also be used.
5. **Enrichment cultures** provide conditions in a broth that enhance the growth of one particular organism in a mixed population.

4.6 Methods to detect and measure bacterial growth

A. Direct cell counts generally do not distinguish between living and dead cells.
1. **Direct microscopic count**
2. The **Coulter counter** and a **flow cytometer** count cells as they pass through a minute aperture.
3. **Viable cell counts**
4. **Plate counts** are based on the fact that an isolated cell will form a single colony.
5. **Membrane filtration** concentrates bacteria by filtration.
6. The **most probable number (MPN) method** is a statistical assay based on the theory of probability and is used to estimate cell numbers.

B. **Measuring biomass**

1. **Turbidity** of a culture is a rapid measurement that can be correlated to the number of cells; a spectrophotometer is used to measure turbidity.
2. **Wet weight** and **dry weight** are proportional to the number of cells in a culture.
3. The **quantity of a cell constituent** such as nitrogen can be used to calculate biomass.

C. **Measuring cell products**

1. **pH indicators** can be used to monitor acid production.
2. **Gas production** can be detected by pH changes or by using an inverted tube in culture media to trap gas.
3. **ATP** is detected by employing luciferase.

4.7 Bacterial growth in laboratory conditions

A. Bacterial growth follows a **growth curve** when they are grown in a closed system.

1. **Lag**—number of cells does not increase
2. **Log**—cells divide at a constant rate
3. **Stationary**—a required nutrient is used up, oxygen is in short supply, or toxic metabolites accumulate
4. **Death**—number of viable cells in the population decreases
5. **Prolonged decline**- gradual decrease in the number of viable cells in the population over a long period of time

B. **Colony growth**: The position of a single cell within a colony markedly determines its environment; cells on the edge may be in log phase whereas those in the center may be in the death phase.

C. **Continuous cultures**: Bacteria can be maintained in a state of continuous exponential growth by using a chemostat.

4.8 Bacterial growth in nature

D. **Mixed populations**: Bacteria often grow in close associations with other kinds of organisms; the metabolic activities of one organism may facilitate the growth of another organism.

E. **Biofilms**: Bacteria may live suspended in an aqueous environment but many attach to surfaces and live as a biofilm, a polysaccharide-encased community.