CHAPTER 10
BACTERIAL GROWTH

WHY IS THIS IMPORTANT?

- Increase in numbers is one of the requirements for infection.
  - This increase is dependent upon bacterial growth.
- Understanding the requirements for growth will help in understanding the infectious process.
- Specific techniques used by clinical microbiologists have a key role in identifying and diagnosing bacterial diseases.

OVERVIEW
**BACTERIAL GROWTH**

- Infectious organisms have specific growth requirements.
- These specific requirements allow for a maximum increase in numbers of infectious organisms.
- Increased numbers of pathogens help to defeat the host defense.

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**BACTERIAL GROWTH**

- Each division of bacteria is called a generation.
- The time between divisions is called generation time.
- Some pathogens have excessively long generation times while others have very short generation times.
- The shorter the generation time, the faster the number of bacteria increases within the host.

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**BACTERIAL GROWTH**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Disease</th>
<th>Optimal Growth Temperature (°C)</th>
<th>Generation Time (Hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Cholera, diseases, opportunistic infections, and urinary tract infections</td>
<td>40</td>
<td>0.35</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Skin, respiratory, and other infections</td>
<td>37</td>
<td>0.47</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Mucocutaneous infections</td>
<td>37</td>
<td>0.58</td>
</tr>
<tr>
<td>Clostridium tetani</td>
<td>Tetanus</td>
<td>37</td>
<td>0.58</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Tuberculosis</td>
<td>37</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Note: From Microbiology: A Clinical Approach © Garland Science
**REQUIREMENTS FOR BACTERIAL GROWTH**

- How well bacteria grow depends on the environment in which the organisms live.
- Growth requirements can be divided into two major categories:
  - Physical
  - Chemical

**PHYSICAL REQUIREMENTS FOR BACTERIAL GROWTH**

- The physical requirements for growth fall into three classifications:
  - Temperature
  - pH
  - Osmotic pressure

**TEMPERATURE**

- Bacteria are found in all ranges of temperatures.
- Bacteria can be separated according to temperature ranges in which they grow best.
  - Psychrophiles – grow at cold temperatures
  - Mesophiles – grow at moderate temperatures
  - Thermophiles – grow at high temperatures
TEMPERATURE

- The minimum growth temperature is the lowest temperature at which an organism grows.
- The maximum growth temperature is the highest temperature at which an organism grows.
- The optimum growth temperature is the temperature at which the highest rate of growth occurs.
  - The optimum growth temperature varies between bacterial types.

Temperature affects growth.
- Increased temperature breaks chemical bonds.
  - This causes changes in the three dimensional structure.
  - These changes can inhibit or destroy the ability for the molecules to function properly.

Variable temperature requirements are seen in certain diseases.
- *Treponema pallidum* (the causative agent of syphilis) likes lower temperatures.
  - Lesions are first seen on exterior parts of the body including lips, tongue, and genitalia.
TEMPERATURE

- *Mycobacterium leprae* (the causative agent of leprosy) also likes lower temperatures.
  - Lesions are first seen on the extremities of the body including the face, ears, hands, feet, and fingers.

pH

- Bacteria grow in a wide range of pH values.
- Most bacteria prefer the neutral pH of 7.0.
- Some bacteria are acidophiles that grow at extremely low pH values.
  - *Helicobacter pylori* (causes stomach and duodenal ulcers) grows at a low pH value but is not an acidophile.

pH

- pH can negatively affect protein structure.
  - An excess of hydrogen ions causes bonds to break.
  - This changes three-dimensional structure.
  - Changes in three-dimensional structure destroy protein function.
  - Destruction of protein function can be a lethal event.
OSMOTIC PRESSURE

- Osmotic pressure is the pressure exerted on bacteria by their environment.
- One of the major agents exerting such pressure is water.
- Osmotic pressure can inhibit bacterial growth.
- High salt concentrations can be used to preserve food (cure meats).
  - Cause a hypertonic environment and plasmolysis.
  - This is an imperfect way to preserve food because some bacteria are halophilic and thrive in high salt concentrations.

OSMOTIC PRESSURE

- Halophilic organisms can be divided into:
  - Obligate – requiring a high salt concentration
  - Extreme – requiring very high levels of salt
  - Facultative – can grow either with or without high salt levels

OSMOTIC PRESSURE

- The human body provides bacteria with the following:
  - Optimal osmotic pressure
  - Optimal temperature range
  - Optimal pH range
- The human body is therefore an excellent incubator for pathogens.
CHEMICAL REQUIREMENTS FOR GROWTH

- Many of the chemical requirements for bacteria are the same as for human cells.
- The chemical requirements are almost as variable as bacterial species themselves.
- Several core chemicals are required for bacterial growth.

CHEMICAL REQUIREMENTS FOR GROWTH

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon, oxygen, hydrogen</td>
<td>Required for cell structures</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Required for making bacterial amino acids and nucleic acids</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Required for making some bacterial amino acids</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Required for making bacterial nucleic acids, membrane phospholipid bilayer, and ATP</td>
</tr>
<tr>
<td>Potassium, magnesium, calcium</td>
<td>Required for functioning of certain bacterial enzymes</td>
</tr>
<tr>
<td>Iron</td>
<td>Required for bacterial metabolism</td>
</tr>
</tbody>
</table>

CARBON

- All biological molecules contain carbon.
- Bacteria are classified based on ways that they acquire carbon.
  - Chemoheterotrophs – obtain carbon by breaking down other carbon molecules
  - Chemoautotrophs – obtain carbon from CO₂
**NITROGEN**

- Nitrogen is involved in protein synthesis.
  - It is an integral part of amino acid structure.
  - It is part of the structure of nucleic acids.
  - It can be obtained in a variety of ways:
    - Decomposition of existing proteins
    - From ammonium ions found in organic materials
    - Nitrogen fixation

**SULFUR**

- Bacteria must have sulfur to make amino acids and some vitamins.
- Sulfur is obtained from decomposition.
- Sulfur can be procured in the sulfate ions (SO$_4^{2-}$) and from H$_2$S.

**PHOSPHOROUS**

- Phosphorus is essential for the synthesis of nucleic acids, AMP, ADP, and ATP.
- It is a major component for the development of the plasma membrane.
- Bacteria obtain phosphorus by cleaving ATP or ADP or from phosphate ions.
**ORGANIC GROWTH FACTORS & TRACE ELEMENTS**

- Bacteria use growth factors such as vitamin B₁, B₂, and B₆.
- Bacteria cannot synthesize these growth factors so they must be obtained from the environment.
- Bacteria also require potassium, magnesium, and calcium as enzyme co-factors.
- Some bacteria also require trace elements such as iron, copper, molybdenum, and zinc.

**OXYGEN**

- Many bacteria do not require oxygen for growth.
- Some die in the presence of oxygen.
  - This is due to the production of the superoxide free radical form of oxygen.
  - This form is unstable and steals electrons from other molecules.
  - This then leads to the death of the organism.

- There are two types of bacteria that grow in the presence of oxygen:
  - Aerobes – require oxygen for growth
  - Facultative anaerobes – can grow with or without oxygen.
  - Both types produce an enzyme called superoxide dismutase that converts free radical oxygen to molecular oxygen and peroxide.
**OXYGEN**

- Peroxide is also poisonous.
- Bacteria produce two enzymes to deal with peroxide:
  - Catalase – converts peroxide to water and oxygen
  - Peroxidase – converts peroxide to water.

**OXYGEN**

- There are three major categories of bacteria based on oxygen use:
  - Obligate aerobes – require oxygen for growth
  - Obligate anaerobes – cannot survive in the presence of oxygen
  - Facultative anaerobes – can grow with or without oxygen.

**OXYGEN**

- There are two additional smaller categories of bacteria based on oxygen use:
  - Aerotolerant – grows in oxygen but does not use it in metabolism
  - Microaerophile – requires only low levels of oxygen for growth.
GROWTH OF ANAEROBIC ORGANISMS

- Special growth media and incubation conditions are required for anaerobic bacteria to grow.
- There are two methods used for culturing anaerobic bacteria.

GROWTH OF ANAEROBIC ORGANISMS

- The first method uses the medium sodium thioglycolate, which forms an oxygen gradient during growth.
  - Aerobic organisms grow at the top.
  - Anaerobic organisms grow at the bottom.
  - Facultative anaerobes grow throughout the medium.

GROWTH OF ANAEROBIC ORGANISMS

- Localized bacterial growth
GROWTH OF ANAEROBIC ORGANISMS

- The second method for growing anaerobic organisms is in a GasPak™ jar
  - This incubation container provides an oxygen-free environment.
  - Only obligate and facultative anaerobes can grow via this method.

GROWTH MEDIA

- Growth media must provide all of the essential growth factors.
- Some bacteria are considered to be fastidious.
  - They require a large number of these growth factors rather than just one or two growth factors.
  - They grow very slowly and can be missed diagnostically.
GROWTH MEDIA

- There are two types of growth media:
  - Chemically defined media
  - Complex media

CHEMICALLY DEFINED MEDIA

- Chemically defined media are those in which the chemical composition is precisely known.
- They are used for the laboratory analysis of compounds produced by specified bacteria.

COMPLEX MEDIA

- Complex media contain not only numerous ingredients of known chemical composition but also digested proteins and extracts derived from plants or meats.
- The exact chemical composition of these digests and extracts is not known.
**COMPLEX MEDIA**

- Complex media are often called a nutrient and are available in two forms:
  - Nutrient agar (solid)
  - Nutrient broth (liquid)

**GROWTH MEDIA: Identifying Pathogens**

- Growth media can be used to identify pathogens in several ways.
- All use selective media and differential media
  - A selective medium is one that contains ingredients that prohibit the growth of some organisms while fostering the growth of others.
  - A differential medium is one that contains ingredients that can differentiate between organisms.
- Many selective media are also differential media.

- Several types of media can identify pathogens.
  - Bismuth sulfate agar only grows *Salmonella enterica* serovar Typhi (causes typhoid fever).
  - There are selective media for the variety of *Neisseria* pathogens.
  - Blood agar media identify production of hemolysins.
    - There are three types of hemolysins: alpha, beta, and gamma.
CHARACTERISTICS OF BACTERIAL GROWTH

- Bacteria divide primarily by binary fission
  - The parent cell divides into two daughter cells.
- Each division is considered a generation.
- The time between divisions is the generation time.
CHARACTERISTICS OF BACTERIAL GROWTH

- Generation times vary between bacterial species and are heavily influenced by:
  - Environmental pH
  - Oxygen level
  - Availability of nutrients
  - Temperature

THE BACTERIAL GROWTH CURVE

- Lag phase – bacteria are adjusting to their environment
  - This varies depending on the organisms and the environment.

THE BACTERIAL GROWTH CURVE

- Log phase – the number of bacteria doubles exponentially
  - There is a constant minimum generation time.
  - This phase lasts only as long as a suitable level of nutrients is available.
  - Bacteria are the most metabolically active and most susceptible to antibiotics.
THE BACTERIAL GROWTH CURVE

- Stationary phase – the number of cells dividing is equal to the number dying
  - It is caused by a decreasing availability of nutrients.

- Death phase (logarithmic decline phase) – a continuous decline in the number of dividing cells
  - It is caused by the exhaustion of the nutrient supply and by a build-up of metabolic waste.

MEASUREMENT OF BACTERIAL GROWTH

- There are two ways to measure bacterial growth:
  - Direct
  - Indirect
DIRECT METHODS OF MEASUREMENT

- Direct methods of measuring bacterial growth include:
  - Direct cell counts
  - Viable cell counts
  - Plate counts
  - Most probable number
  - Membrane filtration

DIRECT METHODS OF MEASUREMENT

- Membrane filtration is used to look for water contamination
  - This method generates the fecal coliform count.
  - The filter pores are small enough to exclude bacteria.
  - Filters are placed on media plates and incubated.
  - The number of contaminating bacteria can then be counted.

INDIRECT METHODS OF MEASUREMENT

- Indirect methods of measuring bacterial growth include:
  - Turbidity – most often used
  - Total weight
  - Chemical constituents
  - Measuring cell products
CLINICAL IMPLICATIONS OF BACTERIAL GROWTH

- Many bacteria are fastidious in a laboratory setting.
- Some bacteria cannot be grown in a laboratory setting.
- Some bacteria have stringent nutritional requirements.
- All of these things can affect diagnosis and treatment of infection.

Bacterial growth requirements can lead to missed identification of pathogens and wrong diagnoses
- This can be caused by improper handling of clinical specimens.
- It can also be caused by improper culturing.
- Specific standard procedures for collecting specimens are intended to limit these problems.