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

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

BACTERIAL GROWTH

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Disease</th>
<th>Optimal growth temperature °C</th>
<th>Generation time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Urinary tract infections</td>
<td>40</td>
<td>0.55</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>Urinary tract infections</td>
<td>37</td>
<td>0.47</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Respiratory infections</td>
<td>37</td>
<td>0.58</td>
</tr>
<tr>
<td>Chlamydia pneumonia</td>
<td>Pneumonia</td>
<td>37</td>
<td>0.58</td>
</tr>
<tr>
<td>Mycobacteria tuberculosis</td>
<td>Tuberculosis</td>
<td>37</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 30.1: Comparison of optimal growth temperature and generation times for several pathogenic bacteria.
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 RANGES FOR BACTERIA

- 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

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- 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.
**TEMPERATURE**

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

- *Mycobacterium leprae* (the causative agent of leprosy) also prefers lower temperatures
  - Lesions are first seen on the cooler 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
**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)
  - Creates 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>Oxygen, Nitrogen</td>
<td>Help components of all organic molecules</td>
</tr>
<tr>
<td>Water</td>
<td>Necessary for making bacterial proteins and adenosine triphosphate</td>
</tr>
<tr>
<td>Minerals</td>
<td>Necessary for enzymatic functions and the nutrition of certain bacterial enzymes</td>
</tr>
<tr>
<td>Iron</td>
<td>Necessary for bacterial nutrition</td>
</tr>
</tbody>
</table>
CARBON

- All biological molecules contain carbon
- Other than water, carbon is the most important chemical requirement for bacterial growth

NITROGEN

- Nitrogen is involved in protein synthesis
  - It is an integral part of amino acid structure
  - It is part of the structure of DNA and RNA
- 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 some amino acids and vitamins
- Sulfur is obtained from decomposition
- Sulfur can be procured in the sulfate ions ($\text{SO}_4^{2-}$) and from $\text{H}_2\text{S}$
PHOSPHORUS

- 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 B
- Bacteria cannot synthesize these growth factors so they must be obtained from the environment
- Bacteria also require potassium, magnesium, and calcium as enzyme cofactors
- 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
OXYGEN

♦ 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

HYDROGEN PEROXIDE

♦ Peroxide is produced by bacteria and is also poisonous
♦ Bacteria produce two enzymes to deal with peroxide:
  ♦ Catalase – converts peroxide to water and oxygen
  ♦ Peroxidase – converts peroxide to just 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
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 can be 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.
- 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.
GROWTH MEDIA: Identifying Pathogens

- Several types of media can identify pathogens
  - Bismuth sulfate agar only grows *Salmonella enterica* serovar Typhi (causes typhoid fever)
  - Blood agar can differentiate *Streptococcus pneumoniae* (alpha hemolysis) from *Streptococcus pyogenes* (beta hemolysis)

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
THE BACTERIAL GROWTH CURVE

- **Lag phase** – bacteria are adjusting to their environment
  - Little, if any, binary fission occurs

- **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

- **Stationary phase** – the number of cells dividing is equal to the number dying
  - It is caused by a decreasing availability of nutrients
THE BACTERIAL GROWTH CURVE

- 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 buildup of metabolic waste

MEASUREMENT OF BACTERIAL GROWTH

- There are two ways to measure bacterial growth:
  - Direct method
  - Indirect method

DIRECT METHODS OF MEASUREMENT

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

**MEMBRANE FILTRATION**

**INDIRECT METHODS OF MEASUREMENT**

- Indirect methods of measuring bacterial growth include:
  - Measuring biomass
  - Turbidity – most often used
  - Total weight
  - Chemical constituents
  - Measuring cell products
  - Acids and gases produced
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

Fastidious bacteria include:
- *Mycobacterium tuberculosis* – grown *in vivo*
- *Haemophilus influenzae* – grown on chocolate agar (made from heated blood)

SPECIMEN COLLECTION

- 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