PHARMACEUTICAL MICROBIOLOGY and IMMUNOLOGY

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OBJECTIVES

- Bacterial growth
- Bacterial colony
- Bacterial spore

Bacterial growth

- Bacterial growth is the **asexual reproduction**, or **cell division**, of a bacterium into two daughter cells, in a process called **binary fission**

- Before cell replication can take place, a variety of chemical reactions must occur in the cell; collectively, these reactions are referred to as **metabolism**

- Metabolic reactions are either energy releasing, called **catabolic reactions**, or energy requiring , called **anabolic reactions**

Bacterial growth

- Several classes of catabolic and anabolic reactions occur in cells

- **Microbial nutrition** is really all about supplying cells with the chemical tools they need to make monomers. These chemical tools are called **nutrients**

- Not all nutrients are required in the same amounts; some nutrients, called **macronutrients**, are required in large amounts, while others, called **micronutrients**, are required in lesser, sometimes even trace amounts

Bacterial growth

- Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), sulfur (S), potassium (K), magnesium (Mg), sodium (Na), calcium (Ca), iron (Fe) are **macronutrients** in nature and in culture media

- Chromium (Cr), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), tungsten (W), vanadium (V), zinc (Zn) are **micronutrients (trace elements)** needed by living organisms

Bacterial growth

- **Growth factors** are organic compounds that, like micronutrients, are required in very small amounts and only by some cells

- Growth factors include vitamins, amino acids, purines and pyrimidines.

- Although most microorganisms are able to synthesize all of these compounds, some microorganisms require one or more of them preformed from the environment

Bacterial growth

Glycolysis: a biochemical pathway in which glucose is fermented yielding energy (ATP) and various fermentation products

Fermentation: anaerobic catabolism in which an organic compound serves as both an electron donor and an electron acceptor and in which ATP is produced by substrate-level phosphorylation

Respiration: the process in which a compound is oxidized with O_2 (or an O_2 substitute) functioning as the terminal electron acceptor, usually accompanied by ATP production by oxidative phosphorylation

Bacterial growth

- The bacterial cell is a synthetic machine that is able to **duplicate** itself
- The synthetic processes of bacterial cell growth involve as many as 2000 chemical reactions of a wide variety of types
- Some of reactions involve energy transformations. Other reactions involve biosynthesis of small molecules-the building blocks of macromolecules-as well as the various cofactors and coenzymes needed for enzymatic reactions

Bacterial growth

- However, the main reactions of cell synthesis are **polymerizations reactions**, the process by which polymers (macromolecules) are made from monomers

- Once polymers are made, the stage is set for the final events of cell growth: assembly of macromolecules and formation of cellular structures such as the cell wall, cytoplasmic membrane, flagella, ribosomes, inclusion bodies, enzyme complexes and etc.

Bacterial growth

Bacterial growth is the **asexual reproduction**, or **cell division**, of a bacterium into two daughter cells, in a process called binary fission

Binary fission:

In bacteria, growth of an individual cell continues until the cell divides into two new cells, a process called binary fission (two cells have arisen from one cell)

Bacterial growth

Binary fission:

- Replication of DNA marks the initiation of the binary fission process

- The process of replicating nucleic acid begins from a replication origin. Following this, a replication bubble is formed, which separates the DNA strands

- Each of the strands then serve as a template for synthesis of the complementary strand. Then, the DNA or genetic material is duplicated

Bacterial growth

Binary fission:

- The second step in binary fission is growth of the parental cell. After duplication of DNA, the parent cell is ready to reproduce by binary fission process
- As a preparatory step, it grows considerably and increases its size
- At the same time, the two circular DNA strands migrate and attach themselves to plasma membrane in different sites

Bacterial growth

Binary fission:

- Following sufficient growth, the cell elongates and starts pulling apart from opposite poles. A division septum is created transversely in the cell

- In short, the cell membrane extends and pinches inwards. During this process, separation of the two chromosomes takes place

- This step is controlled by a group of proteins that assemble near the division site

Bacterial growth

Binary fission:

- Inward growth of the cell membrane is associated with development of a new cell wall
- The final binary fission step is splitting of the parental cell into two daughter cells, each having a nuclear material (chromosome) of its own
- After binary fission is over, the outcome is two genetically identical daughter cells. Under favorable environmental conditions, each of these cells grow and develop into matured cells

Movie time

Bacterial growth

- During the cell division cycle, all the structural components of the cell double

- The interval for the formation of two cells from one cell is called **generation**, and the time required for this occur is called the **generation time** (**generation time**: the time required for the cell population to double)

- Generation times vary widely among microorganisms. Many bacteria have generation times of 1-3 h, but a few very rapidly growing microorganisms are known that divide in as little as 10 min or as long as several days

Bacterial growth

- Bacteria need nutrients for energy, water to stay hydrated, and a place to grow that meets their environmental preferences
- The ideal conditions vary among types of bacterium
- A **culture medium** is a solid, liquid or semi-solid designed to support the growth of microorganisms

Bacterial growth

- One method of bacterial culture is **liquid culture**, in which the desired bacteria are suspended in a liquid nutrient medium (**broth cultures**)
- When bacteria are grown in broths, they may exhibit patterns of growth ranging from a **sediment at the bottom of the tube, turbid growth throughout the tube or a pellicle** (thick growth at the top of the tube).

Bacterial growth

- Bacteria grow on **solid media** as colonies. A **colony** is defined as a visible mass of microorganisms all originating from a single mother cell, therefore a colony constitutes a clone of bacteria all genetically alike

- These are the characteristics used to describe the morphology of a bacterial colony: size, shape, color (also known as pigmentation), texture, height edge…

Bacterial growth

Bacterial colonies can be M (mucoid), S (smooth) or R (rough):

- **M colony** appears water-like, glistening, and confluent and is a character of a bacterial colony that produces slime or a capsule

- **S colony** is recognized by their moist nature, rounded surface

Bacterial growth

- **R colonies** are rough, dry, granulated, and mutant types of bacteria that lack most of the surface proteins including the capsule and lipopolysaccharides

The ability to show variations in both smooth-rough (S-R) ways and from rough to smooth (R-S) colonies has also been observed in bacteria

Bacterial growth

- Environmental factors influence rate of bacterial growth such as acidity (pH), temperature, water activity, macro and micro nutrients, oxygen levels

- Bacteria have optimal growth conditions under which they thrive, but once outside of those conditions the stress can result in either reduced or stalled growth, dormancy (such as formation spores), or death

Bacterial growth

Temperature :

Bacteria have a minimum, optimum, and maximum temperature for growth. Depending on temperature, bacteria can be classified as:

- **Psychrophile:** bacteria with a growth temperature optimum of 15 ^{0}C or lower and a maximum growth temperature below 20 ^{0}C (psychrophiles are cold-loving bacteria)

- **Psychrotolerant:** bacteria capable of growth at low temperatures but whose growth temperature are above 20 ^{0}C

Bacterial growth

Temperature :

- **Mesophile:** bacteria that grow best at temperatures between 20 and 45 ^{0}C. (most bacteria are mesophilic and include common soil bacteria and bacteria that live in and on the body)

- **Thermophile:** bacteria whose growth temperature optimum are above 45 ^{0}C and 80 ^{0}C (thermophiles are heat-loving bacteria)

Bacterial growth

Oxygen requirements:

Bacteria vary in their need for, or tolerance of oxygen. They can be classified into several groups depending on the effect of oxygen:

Aerobe: bacteria that can use O_2 in respiration (aerobes are capable of growth at full oxygen tensions (air is 21% O_2). They obtain their energy through aerobic respiration

Bacterial growth

Oxygen requirements:

Microaerophile: bacteria that can grow only when oxygen tensions are reduced from that in air. (microaerophiles require a low concentration of oxygen (2% to 10%) for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration

Obligate anaerobe: bacteria that can not use O_2 in respiration and whose growth may be inhibited by O_2. (obligate anaerobes grow only in the absence of oxygen. They obtain their energy through anaerobic respiration or fermentation

Bacterial growth

Oxygen requirements:

Aerotolerant anaerobe: bacteria unable to respire molecular oxygen (O_2) but whose growth are unaffected by the presence of O_2 (aerotolerant anaerobes can't use oxygen to transform energy but can grow in its presence. They obtain energy only by fermentation and are known as obligate fermenters)

Facultative anaerobe: bacteria that grow with or without oxygen, but generally better with oxygen. They obtain their energy through aerobic respiration if oxygen is present, but use fermentation or anaerobic respiration if it is absent

Bacterial growth

pH:

The **optimum growth pH** is the most favorable pH for the growth of bacteria. The lowest pH value that bacteria can tolerate is called the **minimum growth pH** and the highest pH is the **maximum growth pH**

- Most natural environments have pH values between 5 and 9, and bacteria with optima in this range are most common. Only a few species can grow at pH values of less than 2 or greater than 10

Bacterial growth

pH:

- Bacteria that grow optimally at pH less than 5.55 are called **Acidophiles**

- Most bacteria are **Neutrophiles**, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7

- Bacteria that grow best at pH between 8.0 and 10.5 are called **Alkaliphiles**

Bacterial growth

Osmosis:

- Osmosis is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration)

- In an **isotonic environment,** both the water and solute concentration are the same inside and outside the cell and water goes into and out of the cell at an equal rate

Bacterial growth

Osmosis:

- If the environment is **hypertonic**, the water concentration is greater inside the cell while the solute concentration is higher outside (the interior of the cell is hypotonic to the surrounding hypertonic environment). Water goes out of the cell.

- In an environment that is hypotonic, the water concentration is greater outside the cell and the solute concentration is higher inside (the interior of the cell is hypertonic to the hypotonic surroundings). Water goes into the cell.

Bacterial growth

Osmosis:

- In an environment that is **hypotonic**, the water concentration is greater outside the cell and the solute concentration is higher inside (the interior of the cell is hypertonic to the hypotonic surroundings). Water goes into the cell.

- Bacteria found in the sea usually have a specific requirement for the sodium ion in addition to growing optimally at the water activity of seawater. Such organisms called **halophiles**. Halophiles are bacteria that thrive in high salt concentrations.

Bacterial growth

The Bacterial Growth Curve

- In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc (2^n, n = the number of generations)

- This is called **exponential growth**. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in nature.

Bacterial growth

The Bacterial Growth Curve

- When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a typical bacterial growth curve

- When bacteria are grown in a closed system (a batch culture-like a test tube) the population of cells almost always exhibits these growth dynamics:

Bacterial growth

- cells initially adjust to the new medium (**lag phase**) until they can start dividing regularly by the process of binary fission (**exponential phase**)

- when their growth becomes limited, the cells stop dividing (**stationary phase**), until eventually they show loss of viability (**death phase**)

Bacterial growth

Four characteristic phases of the growth cycle are recognized:

1. **Lag Phase**:

- Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged

- Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity

Bacterial growth

1. **Lag Phase**:

- The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum, time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium

Bacterial growth

2. Exponential (log) Phase:

- The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression

- The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as generation time, also the doubling time of the bacterial population

Bacterial growth

3. Stationary Phase:

- Exponential growth cannot be continued forever in a batch culture (e.g. a closed system such as a test tube or flask)

- Population growth is limited by one of three factors:
    1. exhaustion of available nutrients
    2. accumulation of inhibitory metabolites or end products
    3. exhaustion of space, in this case called a lack of "biological space"

Bacterial growth

3. Stationary Phase:

- During the stationary phase, if viable cells are being counted, it can not be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing

- Bacteria that produce secondary metabolites, such as antibiotics, do so during the stationary phase of the growth cycle

(Secondary metabolites are defined as metabolites produced after the active stage of growth)

Bacterial growth

3. Stationary Phase:

- It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process

Bacterial growth

4. Death Phase:

- If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines

- During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase

Bacterial spore

- Certain species of bacteria produce special structures called **endospores** within their cells during a process called **sporulation**

- Endospores are differentiated cells that are very resistant to heat and can not be destroyed easily, even by harsh chemicals

- Endospore forming bacteria are found most commonly in the soil

- The genera *Bacillus* and *Clostridium* are the best studied of endospore-forming bacteria. For example; spores of tetanus and anthrax can survive in the soil for many years

Bacterial spore

- When the bacteria is starving or senses a change in the environment, like extreme temperatures and drought, they will produce a spore
- This spore is a protective, thick cell wall and can help the bacteria survive for several years by living in a dormant state
- The spore is made up of few things but includes peptidoglycan, cytoplasm, water, and bacterial DNA
- Once environmental conditions improve, the spore will break

Bacterial spore

- Endospores (so called because the spore is formed within the cell) are readily seen under the light microscope

- Spores are very impermeable to dyes, so occasionally they are seen as unstained regions within cells that have been stained with basic dyes such as methylene blue

Bacterial spore

Structure of spore

- The spore is structurally much more complex in that it has many layers that are absent from the vegetative cell

- The outermost layer is the **exosporium**, a thin, delicate covering made of **protein**

- Within this are the spore coats, composed of layers of spore-specific proteins

Bacterial spore

Structure of spore

- The spore coat, which acts like a sieve that excludes large toxic molecules like lysozyme, is resistant to many toxic molecules and may also contain enzymes that are involved in germination

- Below the spore coat is the **cortex**, which consists of **loosely cross-linked peptidoglycan**

Bacterial spore

Structure of spore

- Inside the cortex is the **core** (**spore protoplast**), which contains the spore chromosomal DNA which is encased in chromatin-like proteins known as SASPs (small acid-soluble spore proteins), that protect the spore DNA from UV radiation and heat. The core also contains normal cell structures, such as ribosomes and other enzymes, but is not metabolically active

Bacterial spore

Structure of spore

- Spore differs structurally from the vegetative cell primarily in the kinds of structures found outside the core wall

- One chemical substance that is characheristic of endospores but not present in vegetative cells is **dipicolinic acid**

Bacterial spore

Structure of spore

- **Dipicolinic acid** has been found in all endospores examined and is located in the **core**
- Spores are also high in **calcium ions**, most of which are combined with **dipicolinic acid**
- Up to 20% of the dry weight of the endospore consists of **calcium dipicolinate** within the core, which is thought to stabilize the DNA
- **Dipicolinic acid** could be responsible for the heat resistance of the spore, and calcium may aid in resistance to heat and oxidizing agents

Movie time

Bacterial spore

Endospore germination

- Although endospores can lie dormant for decades, they can revert back to a vegetative cell very rapidly
- **Activation** of the process may occur through removal of the stress-inducer that initiated sporulation
- During **germination** loss of resistance properties occurs along with a loss of calcium dipicolinate and cortex components, and degradation of the core-specific proteins

Bacterial spore

Endosporе germination

- **Outgrowth** occurs, involving water uptake and synthesis of new RNA, proteins and DNA until eventually, after a matter of minutes, the vegetative cell emerges from the fractured spore coat and begins to divide again

Bacterial spore

- The **position of the endospore** differs among bacterial species and is useful in identification
- The main types within the cell are **terminal**, **subterminal**, and **centrally placed endospores**
- **Terminal endospores** are seen at the poles of cells, whereas **central endospores** are more or less in the middle. **Subterminal endospores** are those between these two extremes, usually seen far enough towards the poles but close enough to the center so as not to be considered either terminal or central

Bacterial spore

- Examples of bacteria having terminal endospores include *Clostridium tetani*, the pathogen that causes the disease tetanus

- Bacteria having a centrally placed endospore include *Bacillus cereus*