Pharmaceutical Microbiology

Lecture 1

28/09/2020

Fundamentals of Microbiology

Introduction

- **Microbiology** is the study of microorganisms, a large and diverse group of microscopic organisms that exist as single cells or cell clusters; it also **includes viruses**, which are microscopic but not cellular.

- Microorganisms have a tremendous impact on all life and the physical and chemical makeup of our planet.

- They are responsible for cycling the chemical elements essential for life, including carbon,oxygen, nitrogen, sulfur, and hydrogen; more photosynthesis is carried out by microorganisms than by green plants.

Bacteria

- Common noun **bacteria**, singular **bacterium**) are a type of biological cell. They constitute a large domain of prokaryotic microorganisms. Typically a few micrometres in length, bacteria have a number of shapes, ranging from spheres to rods and spirals.
- Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust.
- Bacteria also live in symbiotic and parasitic relationships with plants and animals. Most bacteria have not been characterised, and only about 27 percent of the bacterial phyla have species that can be grown in the laboratory . The study of bacteria is known as bacteriology, a branch of microbiology.

Bacterial shapes

- There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water. There are approximately 5×10^{30} bacteria on Earth, forming a **biomass which exceeds that of all plants and animals.**
- Bacteria are vital in many stages of the nutrient cycle by recycling nutrients such as the fixation of nitrogen from the atmosphere. The thrive inside rocks up to nutrient cycle includes the decomposition of dead bodies; bacteria are responsible for the putrefaction stage in this process.

- There are approximately 39 trillion bacterial cells in the human microbiota as personified by a "reference" 70 kg male 170 cm tall.
- The largest number exist in the gut flora, and a large number on the skin.
- The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, though many are beneficial, particularly in the gut flora.
- However, several species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy, and bubonic plague. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people per year, mostly in sub-Saharan Africa.

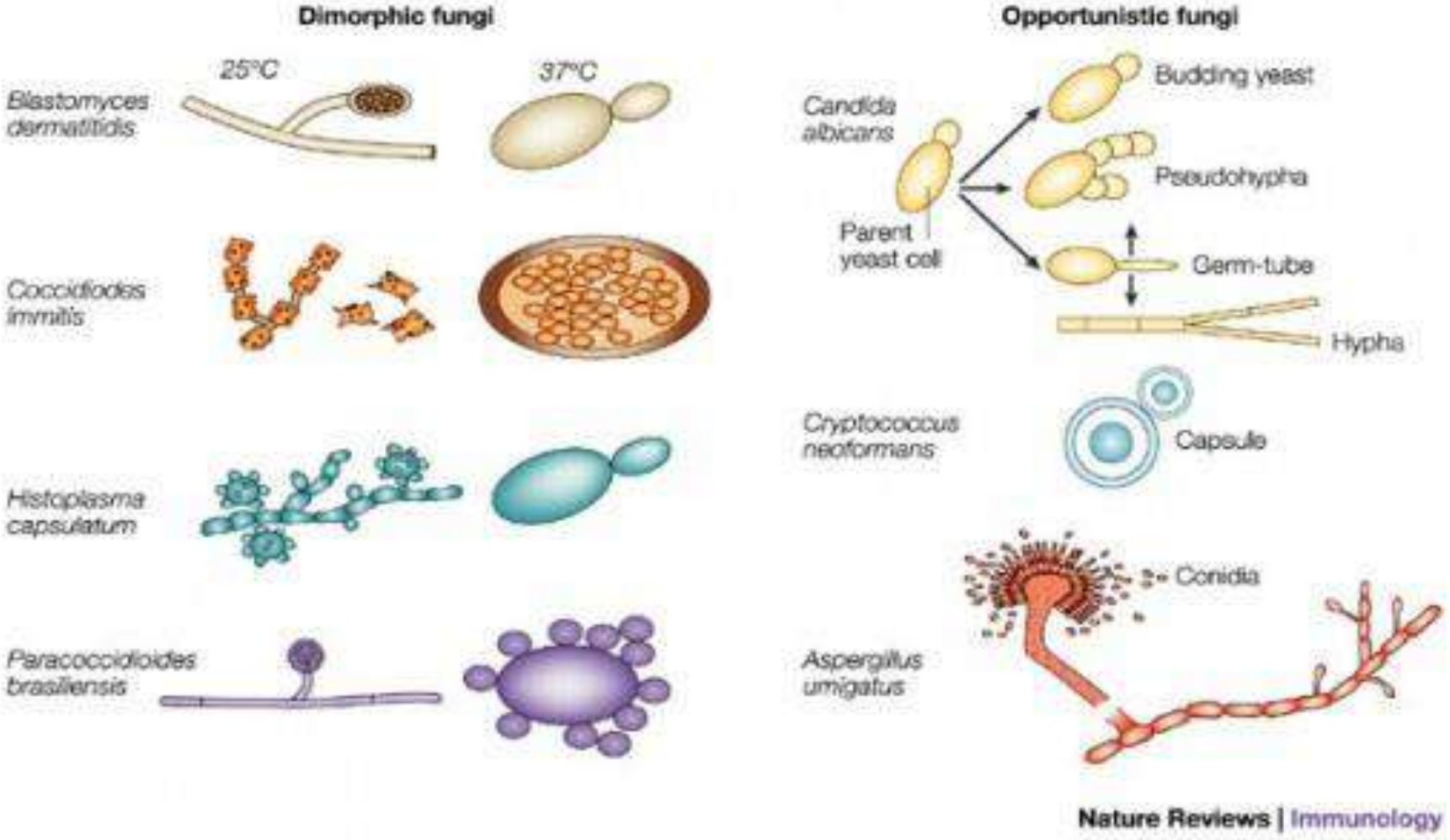
- In developed countries, antibiotics are used to treat bacterial infections and are also used in farming, making antibiotic resistance a growing problem.
- In industry, bacteria are important in sewage treatment and the breakdown of oil spills, the production of cheese and yogurt through fermentation, the recovery of gold, palladium, copper and other metals in the mining sector, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.

Bacteria infection

Fungi

- The fungi are nonphotosynthetic protists growing as a mass of branching, interlacing filaments ("hyphae") known as a mycelium.
- The entire organism is thus a coenocyte (a multinucleated mass of continuous cytoplasm) confined within a series of branching tubes. These tubes, **made of polysaccharides such as chitin, are homologous with cell walls.**
- The mycelial forms are called molds; a few types, yeasts, do not form a mycelium but are easily recognized as fungi by the nature of their sexual reproductive processes and by the presence of transitional forms.

Fungi shapes



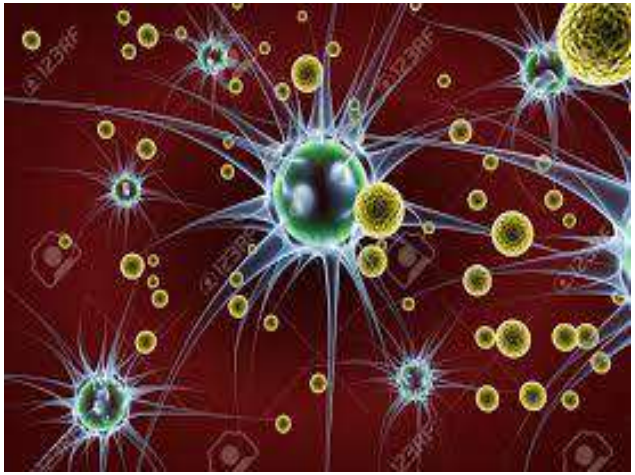
Fungal skin pictures

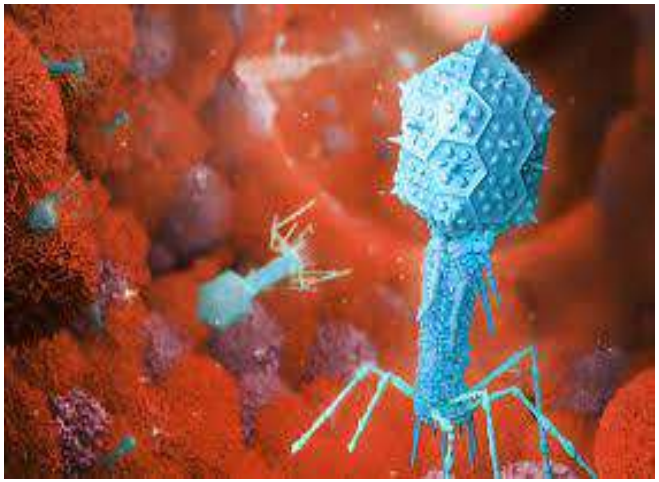
Viruses

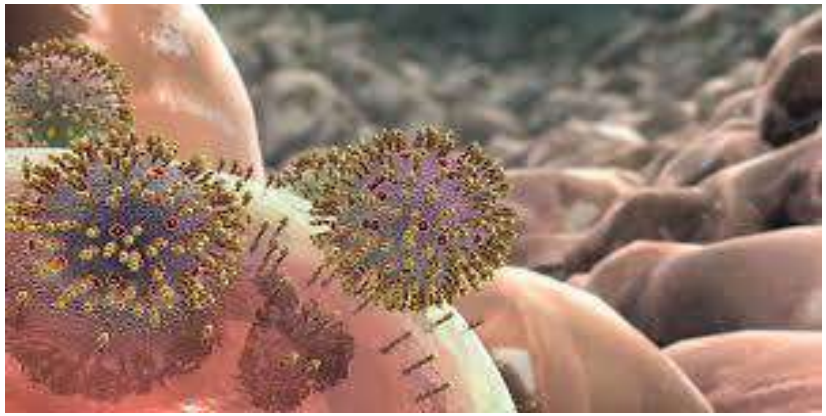
- **Viruses lack many of the attributes of cells, including the ability to replicate**. Only when it infects a cell does a virus acquire the key attribute of a living system— reproduction.
- Viruses are known to **infect all cells, including microbial cells.** Recently, **viruses called virophages have been discovered that infect other viruses.**
- **Viral particles are generally small** (eg, **adenovirus is 90 nm**) and consist of **a nucleic acid molecule, either DNA or RNA, enclosed in a protein coat, or capsid** (sometimes itself enclosed by an envelope of lipids, proteins, and carbohydrates).

- **Proteins—frequently glycoproteins—in the capsid determine the specificity of interaction of a virus with its host cell.**
- The **capsid protects the nucleic acid and facilitates attachment and penetration of the host cell by the virus**. Inside the cell, viral nucleic acid redirects the host's enzymatic machinery to functions associated with replication of the virus.
- **In some cases, genetic information from the virus can be incorporated as DNA into a host chromosome.**

Virus pictures in human



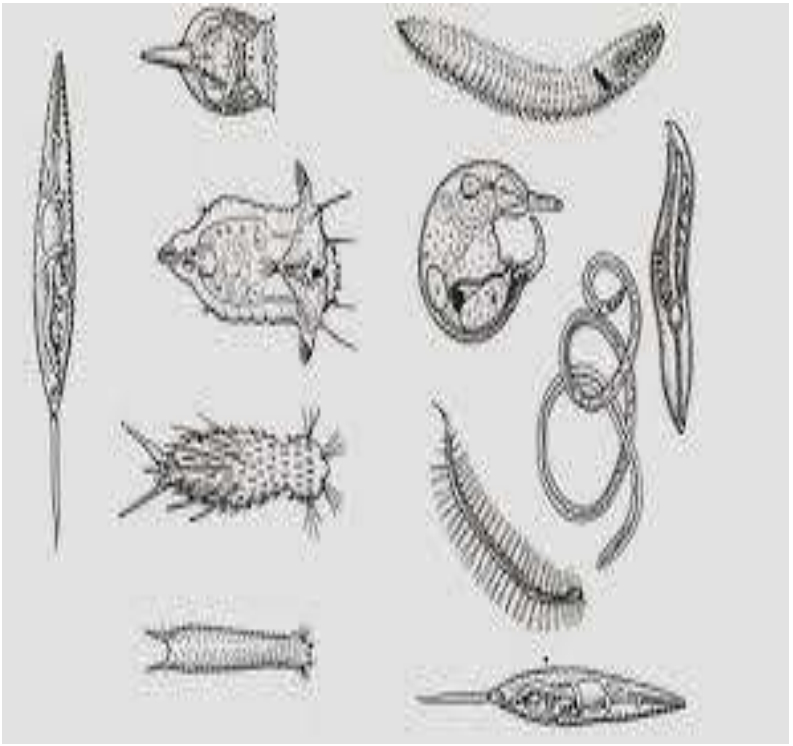


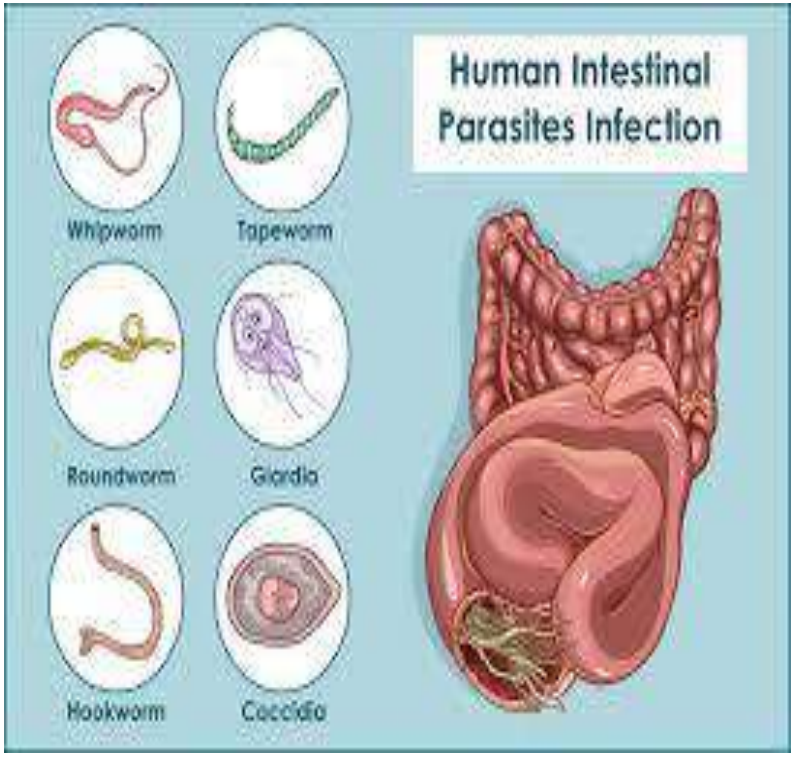


Parasite

- **Human parasites** include **various protozoa and worms that may infect humans that cause parasitic diseases.**
- Human parasites are divided into **endoparasites**, which **cause infection inside the body**, and **ectoparasites**, which **cause infection superficially within the skin.**
- The **cysts and eggs of endoparasites may be found in feces, which aids in the detection of the parasite in the human host** while also **providing the means for the parasitic species to exit the current host and enter other hosts**.

Parasite in humans





Lecture 2

01/10/2020

Classification of Bacteria

- **Taxonomic ranks** form the basis for the organization of bacteria. **Linnaean taxonomy** is the system most familiar to biologists. It uses the formal taxonomic ranks of **kingdom, phylum, class, order, family, genus, and species.**

Taxonomic ranks Formal rank example

- **Kingdom**: Prokaryotae
- **Division**: Gracilicutes
- **Class:** Scotobacteria
- **Order:** Eubacteriales
- **Family:** Enterobacteriaceae
- **Genus:***Escherichia*
- **Species:** *coli*
- **Subtype:** *Escherichia coli* O157: H7

Growth on Media

- **In contrast to viruses and most parasites**, many **bacterial pathogens can be isolated on solid agar-containing media.** The general **cultivation of most bacteria requires media rich in metabolic nutrients.**
- These media generally include **agar, a carbon source, and an acid hydrolysate or enzymatically degraded source of biologic material (eg, casein).** Because of the undefined composition of the latter, these types of media are referred to as **complex media**.

- **Clinical samples** from normally **nonsterile sites** (eg, the throat or the colon) contain multiple species of organisms, including **potential pathogens and resident microbial flora**. Media can be **nonselective** or **selective**; the latter are used to distinguish among the various bacteria in a clinical sample containing many different organisms.

A. Nonselective Media

- **Blood agar** and **chocolate agar** are examples of complex, nonselective media, which support the growth of many different bacteria.
- These media are intended to cultivate as many species as possible, thus giving rise to numerous types of bacterial colonies.

B. Selective Media

- **Because of the diversity of microorganisms** that typically reside at some sampling sites (eg, **the skin, respiratory tract, intestines, vagina**), **selective media are used to eliminate (or reduce) the large numbers of irrelevant bacteria in these specimens**.
- The **basis for selective media is the incorporation of an inhibitory agent that specifically selects against the growth of irrelevant bacteria.**
- Examples of such agents are:
- **Sodium azide**—selects for gram-positive bacteria over gram-negative bacteria
-

Bile salts (sodium deoxycholate)—select for gram-negative enteric bacteria and inhibit gram-negative mucosal and most gram-positive bacteria

- **Colistin and nalidixic acid**—inhibit the growth of many gram-negative bacteria

- Examples of selective media are **MacConkey agar** (**contains bile**) that selects for the Enterobacteriaceae and **CNA blood agar** (contains colistin and nalidixic acid) that selects **for Staphylococci and Streptococci**.

C. Differential Media

- **Upon culture**, some bacteria produce characteristic pigments, and others can be differentiated on the basis of their complement of extracellular enzymes; the activity of these enzymes often can be detected as zones of clearing surrounding colonies grown in the presence of insoluble substrates (e g, **zones of hemolysis in agar medium containing red blood cells).**

- Many of the members of the **Enterobacteriaceae** can be differentiated on the **basis of their ability to metabolize lactose**. For example, whereas pathogenic salmonellae and shigellae **do not ferment lactose on a MacConkey plate form white colonies**, **lactose-fermenting members of the Enterobacteriaceae (eg, *E coli*)** form red or pink colonies.

- However, it should be noted **that biochemical identification is an important means to classify microbial pathogens**.

Chapter 1

Lecture 3

By Dr. Mohammed Hussein Taleb

Bacterial Microscopy

- Historically, the Gram stain, together with visualization by light microscopy, has been among **the most informative methods for classifying the eubacteria.**
- This **staining technique broadly divides bacteria on the basis of fundamental differences in the structure of their cell walls**

- This **typically represents the first step in identifying individual microbial specimens (eg, are they gram negative or gram positive) grown in culture or even directly from patient specimens (eg, urine specimens).**

Biochemical Tests

- Tests such as the **oxidase test**, which uses an artificial electron acceptor, can be used to distinguish organisms on the **basis of the presence or absence of a respiratory enzyme**, **cytochrome C**, the lack of which differentiates **the Enterobacteriaceae from other gram-negative rods**.

- Similarly, **catalase** activity can be used, for example, to differentiate between the gram-positive cocci; the **species staphylococci are catalase positive** whereas the **species streptococci are catalase negative**. If the organism is demonstrated to be catalase positive (*Staphylococcus* spp.), the species can be **subdivided by a coagulase test into *Staphylococcus aureus* (coagulase positive) or *Staphylococcus epidermitidis* (coagulase negative)**

Immunologic Tests—Serotypes, Serogroups, and Serovars

- The designation **"sero"** simply indicates the use of antibodies (polyclonal or monoclonal) that react with specific bacterial cell surface structures such as lipopolysaccharide (LPS), flagella, or capsular antigens.

- The terms "serotype," "serogroups," and "serovars" are, for all practical purposes, identical—they all use the specificity of these antibodies to subdivide strains of a particular bacterial species.

- This has been described earlier in this chapter as it relates to the relationship *E coli* O157:H7 and HUS.

Phases of the Microbial Growth Curve

- Lag Zero
- Exponential Constant
- Maximum stationary Zero
- Decline Negative (death)

Curve of bacterial growth

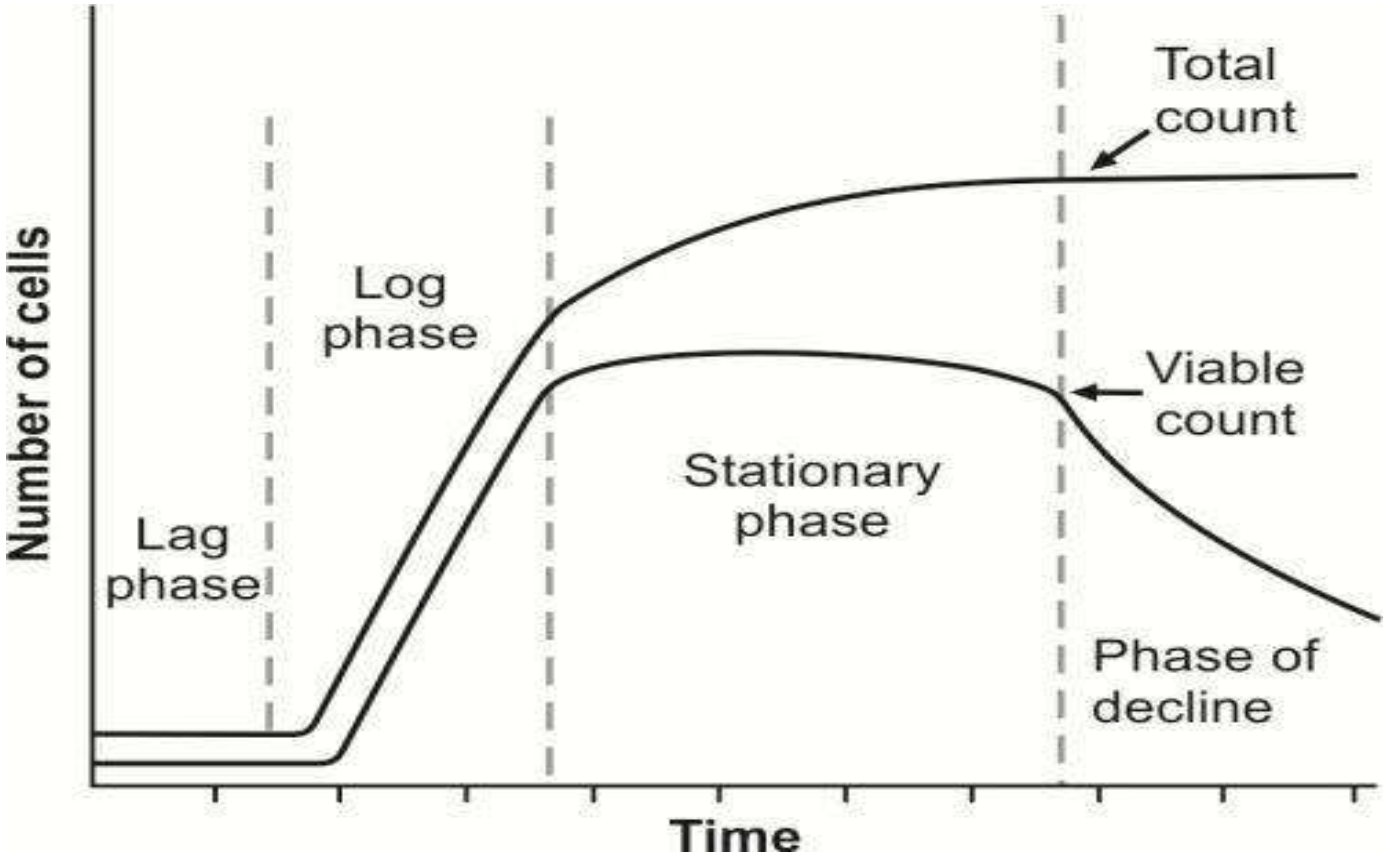


Figure 4-2

1-The Lag Phase

The zero phase

- The lag phase represents a period during which cells, depleted of metabolites and enzymes as the result of the unfavorable conditions that existed at the end of their previous culture history, adapt to their new environment.

- Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume.

- If the cells are taken from an entirely different medium, it often happens that they are genetically incapable of growth in the new medium.

- In such cases, a long lag in growth may occur, representing the period necessary for a few mutants in the inoculum to multiply sufficiently for a net increase in cell number to be apparent.

The Exponential Phase

- The log phase
- During the exponential phase, the cells are in a steady state. **New cell material is being synthesized at a constant rate, but the new material is itself catalytic, and the mass increases in an exponential manner.**

The Maximum Stationary Phase

- Eventually, the exhaustion of nutrients or the accumulation of toxic products causes growth to cease completely. In most cases, however, cell turnover takes place in the stationary phase:
- There is a slow loss of cells through death, which is balanced by the formation of new cells through growth and division.
- When this occurs, the total cell count slowly increases, although the viable count stays constant.

The Death Phase

- **The Phase of Decline**
- After a period of time in the stationary phase, which varies with **the organism and with the culture conditions, the death rate increases until it reaches a steady level. the rate of cell death is much slower than that of exponential growth.**
- Frequently, after **the majority of cells have died, the death rate decreases, so that a small numbe of survivors may persist for months or even years**. This **persistence may in some cases reflect cell turnover.**

ANTIMICROBIAL AGENTS

- Definitions
- The following terms are commonly used in connection with antimicrobial agents and their uses.

A. Biocide

- A chemical or physical agent, usually broad spectrum, that inactivates microorganisms.
- Chemical biocides include hydrogen peroxide, alcohols, bleach, cycloheximide, and phenols, and physical biocides include heat and radiation. Biocides are generally broad spectrum, in contrast to anti-infectives, which have a narrower range of antimicrobial activity.

B. Bacteriostatic

- A specific term referring to the property by which a biocide is able to inhibit bacterial multiplication; upon removal of the agent, multiplication resumes. (The terms "fungistatic" an "sporostatic" refer to biocides that inhibit the growth of fungi and spores, respectively.)

- ## C. Bactericidal

- A specific term referring to the property by which a biocide is able to kill bacteria. Bactericidal action differs from bacteriostasis only in being irreversible (ie, the "killed" organism can no longer reproduce even after being removed from contact with the agent). (The terms "fungicidal," "sporicidal," and "virucidal" refer to the property whereby biocides are able to kill fungi, spores, an viruses, respectively.)

- **D. Sterilization**
- A defined process used to render a surface or product free from viable organisms, including bacterial spores.
- **E. Disinfectants**
- **Products or biocides used to reduce the number of viable microorganisms, or bioburden, on or in a product or surface to a level previously specified as appropriate for its intended further handling or use.**
- **Disinfectants are not necessarily sporicidal but are sporostatic, inhibiting germination or outgrowth.**

- **F. Septic**
- Characterized by the presence of pathogenic microbes in living tissues or associated fluids.
- **G. Antiseptic**
- A biocide or product that destroys or inhibits the growth of microorganisms in or on living tissue (eg, skin) or biologic fluids (eg, mucosal secretions).
- **H. Aseptic**
- Free of, or using methods to keep free of, microorganisms.
- **I. Preservation**
- The prevention of multiplication of microorganisms in formulated products, including pharmaceuticals and foods.
- **J. Antibiotics**
- Naturally occurring and synthetically derived organic compounds that inhibit or destroy selective bacteria, generally at low concentrations

Modes of Action

- A. Damage to DNA
- B. Protein Denaturation
- C. Disruption of the Cell Membrane or cell Wall
- D. Disruption of Free Sulfhydryl Groups
- E. Chemical Antagonism