

Introduction

Microbiology is the study of microorganisms (a large and diverse group of microscopic organisms that exist as single cells or cell clusters).

Organisms are classified to two major groups "Prokaryotes and Eukaryotes", based on whether or not the cells have a nucleus (that is, an internal membrane-enclosed region that contains the genetic material). Cells that have a well-defined nucleus are called eukaryotic, whereas cells that lack a nucleus are called prokaryotic. Prokaryotic organisms include bacteria, whereas eukaryotic organisms include fungi, protozoa, and helminthes as well as humans.

Cells of prokaryotic and eukaryotic organisms differ in several significant structural features as illustrated in Figure 1.

CHARACTERISTIC	PROKARYOTIC CELLS	EUKARYOTIC CELLS
Chromosome	Usually single, circular ¹	Multiple
Nucleus	No nuclear envelope or nucleoli	Membrane bound, nucleoli present
Membrane-bound organelles	Not present	Present (examples include mitochondria and endoplasmic reticulum)
Cell wall	Usually present, many contain peptidoglycan	Present in plant cells, no peptidoglycan
Plasma membrane	No carbohydrates, most lack sterols	Sterols and carbohydrates present
Ribosome	70S	80S (70S in organelles)
Average size	0.2–2 mm in diameter	10–100 mm in diameter

Figure 1: Differences between Prokaryotes and Eukaryotes

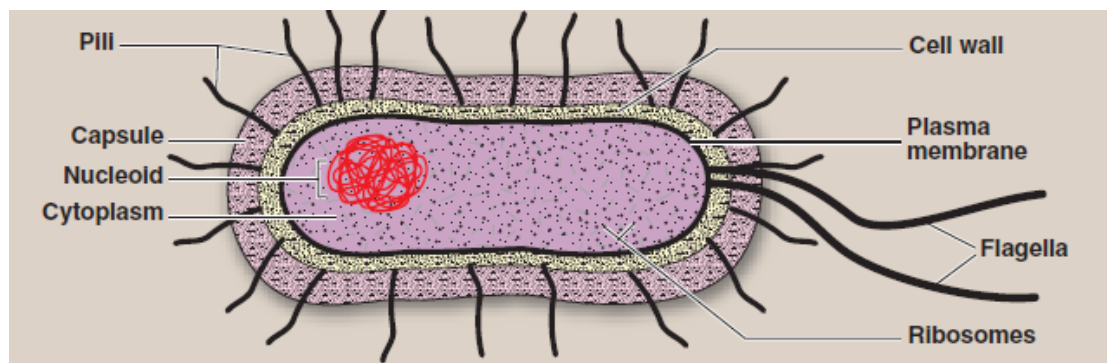
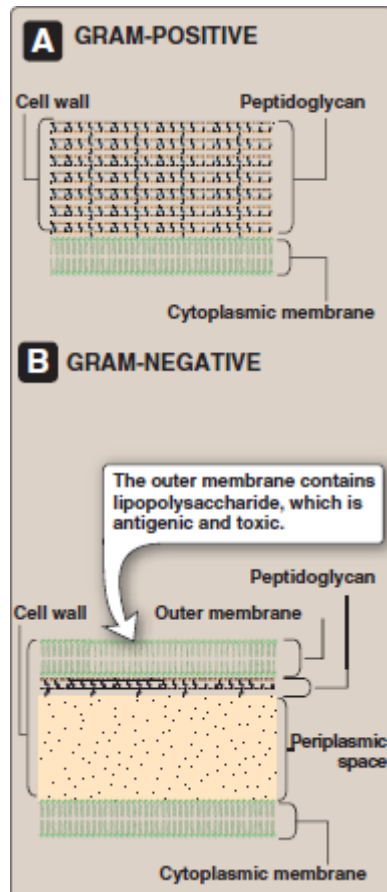


Figure 2: Generalized structure of a bacterial cell.



The cytoplasmic membrane is composed of phospholipid, the molecules of which form two parallel surfaces (called a lipid bilayer) such that the polar phosphate groups are on the outside of the bilayer and the nonpolar lipid chains are on the inside. The membrane acts as a permeability barrier, restricting the kind and amount of molecules that enter and leave the cell.

The peptidoglycan layer determines the shape of the cell. It is composed of a cross-linked polymeric mesh. The glycan portion is a linear polymer of alternating monosaccharide subunits: N acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). This polymer is the carbohydrate “backbone” of the mesh. The “peptido” portion of the polymer is a short string of amino acids that serves to cross-link adjacent polysaccharide strands at the NAM subunits of the backbone, forming a network with high tensile strength

Figure 3: Comparison of gram-positive and gram-negative bacterial cell walls.

Normal Flora

Microorganisms can populate the healthy human body as "normal flora" and even as participants in bodily functions.

BENEFICIAL FUNCTIONS OF NORMAL FLORA

First, the sheer number of harmless bacteria in the lower bowel and mouth make it unlikely that, in a healthy person, an invading pathogen could compete for nutrients and receptor sites.

Second, some bacteria of the bowel produce antimicrobial substances to which the producers themselves are not susceptible.

Third, bacterial colonization of a newborn infant acts as a powerful stimulus for the development of the immune system.

Fourth, bacteria of the gut provide important nutrients, such as vitamin K, and aid in digestion and absorption of nutrients. [Note: Although humans can obtain vitamin K from food sources, bacteria can be an important supplemental source if nutrition is impaired.]

HARMFUL EFFECTS OF NORMAL FLORA

Clinical problems caused by normal flora arise in the following ways:

- 1) The organisms are displaced from their normal site in the body to an abnormal site. An example the introduction of the normal skin bacterium, *S. epidermidis*, into the bloodstream where it can colonize catheters and heart valves, resulting in bacterial endocarditis.
- 2) Potential pathogens gain a competitive advantage due to diminished populations of harmless competitors. For example, when normal bowel flora are depleted by antibiotic therapy leading to overgrowth by the resistant *Clostridium difficile*, which can cause severe colitis.
- 3) Harmless, commonly ingested food substances are converted into carcinogenic derivatives by bacteria in the colon.
- 4) When individuals are immunocompromised, normal flora can overgrow and become pathogenic. [Note: Colonization by normal, but potentially harmful, flora should be distinguished from the carrier state in which a true pathogen is carried by a healthy (asymptomatic) individual and passed to other individuals where it results in disease. Typhoid fever is an example of a disease that can be acquired from a carrier]

Growth and Metabolism

Stages of the bacterial growth cycle: Because bacteria reproduce by binary fission (one becomes two, two become four, etc.), the number of cells increases exponentially with time (the exponential, or log, phase of growth). Depending on the species, the minimum doubling time can be as short as 10 minutes or as long as several days. Eventually, growth slows and ceases entirely (stationary phase) as nutrients are depleted, and toxic waste products accumulate. Most cells in a stationary phase are not dead, however. If they are diluted into fresh growth medium, exponential growth will resume after a lag phase.

Energy production

Depending on the biochemical mechanism used, bacterial metabolism can be categorized into three types: aerobic respiration, anaerobic respiration, and fermentation.

1. Aerobic respiration is the metabolic process in which molecular oxygen serves as the terminal electron acceptor of the electron transport chain. In this process, oxygen is reduced to water. Respiration is the energy-generating mode used by all aerobic bacteria.
2. Anaerobic respiration is the metabolic process in which inorganic compounds other than molecular oxygen serve as the terminal electron acceptors. Depending on the species, acceptors can be molecules such as nitrate or sulfate. Anaerobic respiration can be used as an alternative to aerobic respiration in some species (facultative organisms), but is

obligatory in other species (some obligate anaerobes). [Note: Other obligate anaerobes use fermentation as the main mode of energy metabolism. This is particularly true among the anaerobic bacteria of medical importance.]

3. Fermentation is an anaerobic process utilized by some bacterial species. It is the metabolic process by which an organic metabolic intermediate derived from a “fermentable” substrate serves as the final electron acceptor. The substrates that can be fermented and the final endproducts depend on the species.

By comparison to aerobic and anaerobic respiration, fermentation yields very little energy. The purpose of fermentation is to recycle nicotinamide adenine dinucleotide hydrogen (NADH) back to NAD.

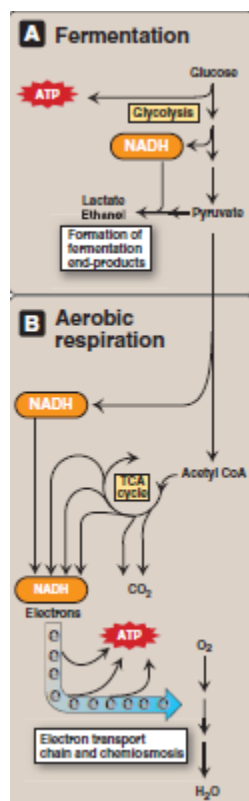


Figure 4: Overview of respiration, fermentation, and energy production in bacteria. [Note: Compounds other than oxygen, such as nitrate and sulfate, can be used as terminal electron acceptors in anaerobic respiration.]

THE BACTERIAL GENOME

The genome often consists of a single chromosome that carries all of the essential genes and one or more varieties of plasmid that generally carry nonessential genes (Figure 5).

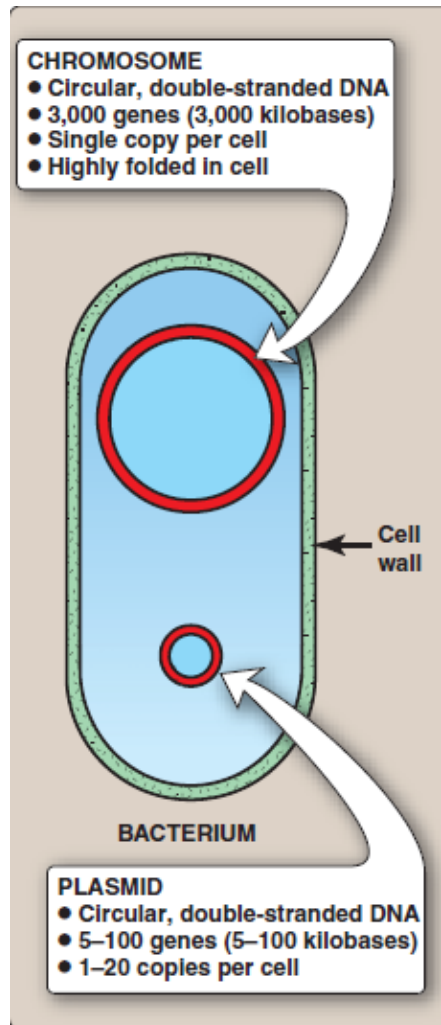


Figure 5: The bacterial genome

A. The chromosome

All of the essential genes and many nonessential genes of the bacterium are generally carried on a single, long piece of circular, double-stranded DNA.

B. Pathogenicity islands

Pathogenicity islands are discrete genetic elements that encode virulence factors, such as toxins, adhesins, secretion systems, and iron transport proteins. These islands, which range in size from 10 to 200 kB, can be horizontally transferred between bacteria, resulting in enhanced virulence and fitness in the recipient.

C. Plasmids

- Small circular double strand DNA, which range in size from 1.5 kilobase (kb) pairs to 120 kb pairs (less than one tenth the size of the bacterial chromosome).
- Plasmids replicate independently of the chromosome and can exist in the cell as one copy or as many copies.
- Plasmids can carry genes that encode toxins or proteins that promote the transfer of the plasmid to other cells but usually do not include genes that are essential for cell growth or replication.
- Many plasmids contain mobile DNA sequences (transposons) that can move between plasmids and between plasmids and the chromosome. Transposons, the repository for many antibiotic resistance genes, are responsible for the ability of some plasmids to integrate into the chromosome.

BACTERIOPHAGE

A bacteriophage (phage) is a virus that replicates inside a bacterial cell.

A. Virulent phage

Infection of a bacterium with a virulent phage results in the death of the cell by lysis, with release of newly replicated phage particles.

B. Temperate phage

A bacterium infected with a temperate phage can have the same fate as a bacterium infected with a virulent phage (lysis rapidly following infection). However, an alternative outcome is also possible: Namely, after entering the cell, the phage DNA, rather than replicating autonomously, can integrate into the chromosome of the host cell. In this state (prophage), the expression of phage genes is repressed indefinitely by a regulatory protein encoded within the phage genome. No new phage

particles are produced, the host cell survives, and the phage DNA replicates as part of the host's chromosome.

GENE TRANSFER

Genes can be transferred from one bacterial cell to another by three distinct mechanisms: conjugation, transduction, and transformation.

A. Conjugation

Conjugation is the process by which bacteria transfer genes from one cell to another by cell-to-cell contact. The donor (male) and recipient (female) cells must have the proper genetic constitution to adhere to each other, and they form a cytoplasmic bridge between the cells through which DNA can pass.

B. Transduction

Transduction refers to transfer of genes from one cell to another via a phage vector without cell-to-cell contact. There are two ways in which this can occur: generalized transduction and specialized transduction. In each case, the transducing phage is a temperate phage, so that the recipient cell survives the phage infection.

C. Transformation

Transformation is the transfer of genes from one cell to another by means of naked DNA.

Pathogenesis and virulence

A pathogenic microorganism is defined as one that is capable of causing disease.

The methods by which bacteria cause disease can be divided into several stages. Pathogenicity of a microorganism depends on its success in completing some or all of these stages.

The terms “virulence” and “pathogenicity” are often used interchangeably. However, virulence can be quantified by how many organisms are required to cause disease in 50 percent of those exposed to the pathogen (ID₅₀, where I = Infectious and D = Dose), or to kill 50 percent of test animals (LD₅₀, where L = Lethal).

The number of organisms required to cause disease varies greatly among pathogenic bacteria. For example, less than 100 *Shigella* cause diarrhea by infecting the gastrointestinal (GI) tract, whereas the infectious dose of *Salmonella* is approximately 100,000 organisms.

The infectious dose of a bacterium depends primarily on its virulence factors. The probability that an infectious disease occurs is influenced by

both the number and virulence of the infecting organisms and the strength of the host immune response opposing infection.

Virulence factors

Virulence factors are those characteristics of a bacterium that enhance its pathogenicity, that is, properties that enable a microorganism to establish itself and replicate on or within a specific host. Some of the more important steps in the infectious process are reviewed below.

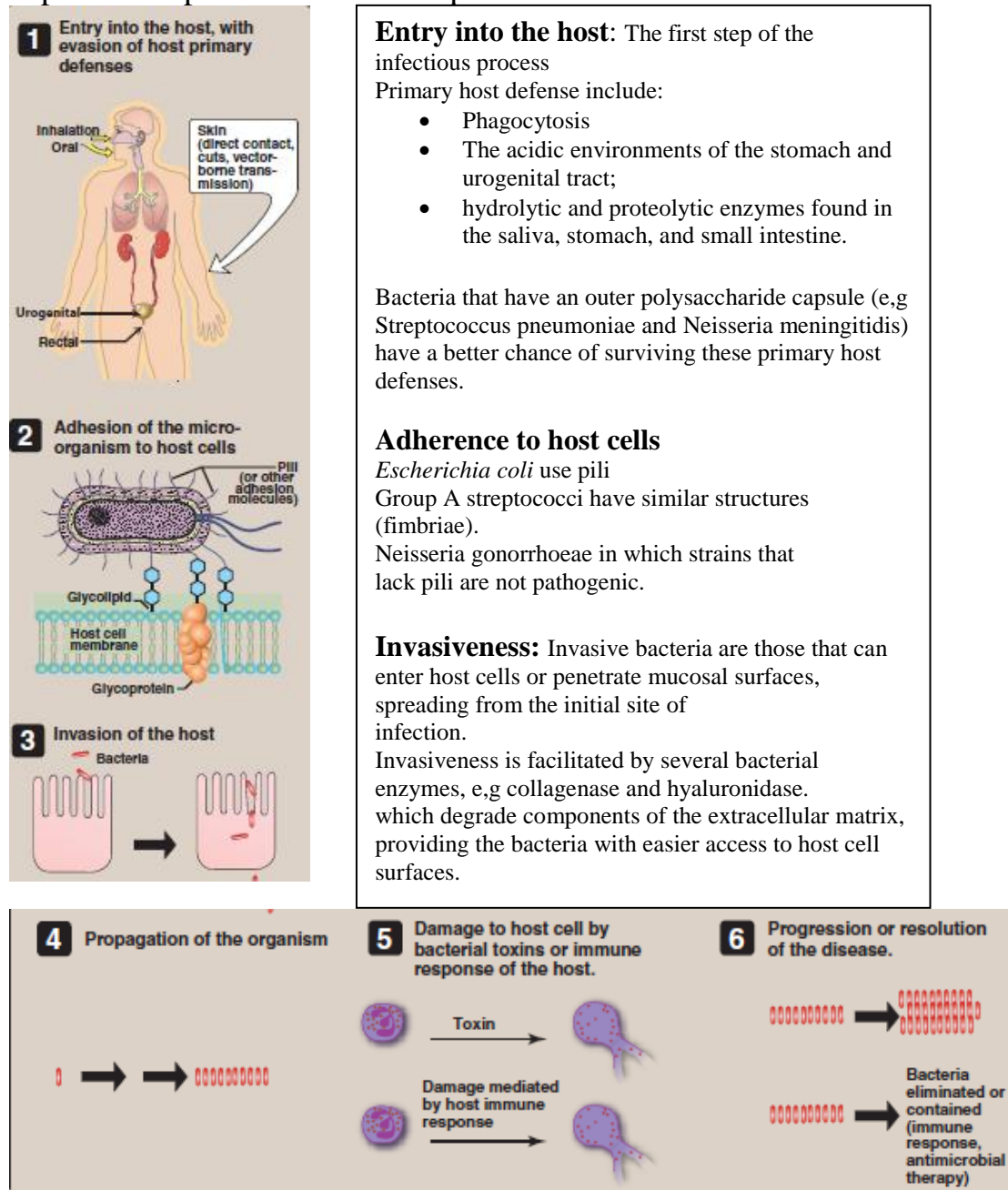


Figure 6: Steps in the infectious process

Invasion is followed by inflammation, which can be either pyogenic (involving pus formation) or granulomatous (having nodular inflammatory lesions), depending on the organism.

The pus of pyogenic inflammations contains mostly neutrophils, whereas granulomatous lesions contain fibroblasts, lymphocytes, and macrophages.

Bacterial toxins: Some bacteria cause disease by producing toxic substances, of which there are two general types: exotoxins and endotoxin. Exotoxins, which are proteins, are secreted by both gram-positive and gram-negative bacteria. In contrast, endotoxin, which is synonymous with lipopolysaccharide (LPS), is not secreted but instead is an integral component of the cell walls of gram-negative bacteria.

Host-mediated pathogenesis

The pathogenesis of many bacterial infections is caused by the host response rather than by bacterial factors. Classic examples of host response-mediated pathogenesis are seen in diseases such as gram-negative bacterial sepsis, tuberculosis, and tuberculoid leprosy. The tissue damage in these infections is caused by various cytokines released from the lymphocytes, macrophages, and polymorphonuclear leukocytes at the site of infection or in the bloodstream. Often the host response is so intense that host tissues are destroyed, allowing remaining bacteria to proliferate.

Antigenic variation

A successful pathogen must evade the host's immune system that recognizes bacterial surface antigens. One important evasive strategy for the pathogen is to change its surface antigens. This is accomplished by several mechanisms. One mechanism, called phase variation, is the genetically reversible ability of certain bacteria to turn off and turn on the expression of genes coding for surface antigens. A second mechanism, called antigenic variation, involves the modification of the gene for an expressed surface antigen by genetic recombination with one of many variable unexpressed DNA sequences. In this manner, the expressed surface antigen can assume many different antigenic structures.

Further Readings:

Lippincott's Illustrated Reviews: Microbiology. Third Edition,

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