Contents

Part 1: Introduction to clinical microbiology

Part 2: Laboratory identification of significant isolates

Part 3: Laboratory diagnosis of infectious diseases: an organ system approach to diagnostic microbiology

*Available online on the Evolve Resources site (http://evolve.elsevier.com/Mahon/microbiology/).

Overview of the microbial world

The study of microorganisms by the Dutch biologist and lens maker Anton van Leeuwenhoek has evolved immensely from its early historic beginnings. Because of Leeuwenhoek's discovery of what he affectionately called *wee beasties* and *animalcules* in a water droplet with his home-made microscope, the scientific community acknowledged him as the "father of protozoology and bacteriology." Today, we know that there are enormous numbers of microbes in, on, and around us in our environment. The vast majority of these microbes do not cause disease. This textbook focuses on microbes that are associated with human disease.

Bacteria

Bacteria are unicellular organisms that are classified as **prokaryotes** (Greek: before kernel [nucleus]). They lack a nuclear membrane and a true nucleus, mitochondria, an endoplasmic reticulum (ER), and Golgi bodies. The absence of the preceding bacterial cell structures differentiates them from **eukaryotes** (Greek *eu:* well or good; Greek *karyon:* kernel). Table 1.1 compares prokaryotic and eukaryotic cell organization; Fig. 1.1 shows both types of cells.

Parasites

Certain eukaryotic parasites (organisms that live at the expense of their hosts) exist as unicellular organisms of microscopic size, whereas others are multicellular organisms. Protozoa are unicellular organisms within the kingdom Protista that obtain their nutrition through ingestion. Some are capable of locomotion (motile), whereas others are non-motile. They are categorized by their locomotive structures: flagella (Latin: whiplike), pseudopodia (Greek: false feet), or cilia (Latin: eyelash). Many multicellular parasites can be quite large; for example, tapeworms may be 7 to 10 m long (see Chapter 28).

Fungi

Fungi are heterotrophic (cannot produce all of its nutrients) eukaryotes that obtain nutrients through absorption. Most fungi are multicellular, and many can reproduce sexually and asexually. Multicellular fungi are composed of filaments called **hyphae** that interweave to form mats called **mycelia**. Yeasts are unicellular fungi that reproduce asexually. "True" yeasts do not form hyphae or mycelia. Molds are filamentous forms that can reproduce asexually and sexually. Certain fungi can assume both morphologies (yeast and hyphae/mycelial

Table 1.1 Comparison of prokaryotic and eukaryotic cell organization

Characteristic	Prokaryote	Eukaryote
Typical size	0.4–2 μm in diameter	10–100 μm in diameter
	0.5–5 μm in length	>10 μm in length
Nucleus	No nuclear membrane; nucleoid region of the cytosol	Membrane-bound nucleus
Genome		
Location	In the nucleoid, at the mesosome	In the nucleus
Chromosomal DNA	Circular; complexed with RNA	Linear; complexed with basic histones and other proteins
Extrachromosomal circular DNA	Plasmids, small circular molecule of DNA in the cytoplasm containing accessory information; most commonly found in gram-negative bacteria; each carries genes for its own replication; can confer resistance to antimicrobial agents	In mitochondria, chloroplasts, and cytoplasm
Reproduction	Asexual (binary fission)	Sexual and asexual
Membrane-bound organelles	Absent	All
Golgi bodies	Absent in all	Present in some
Lysosomes	Absent in all	Present in some; contain hydrolytic enzymes
Endoplasmic reticulum	Absent in all	Present in all; lipid synthesis, transport
Mitochondria	Absent in all	Present in most
Chloroplasts for photosynthesis	Absent in all	Present in algae and plants
Ribosomes: site of protein synthesis (nonmembranous)	Present in all	Present in all
Size	70 S consisting of 50 S and 30 S subunits	80 S consisting of 60 S and 40 S subunits
Electron transport for energy	In the cell membrane; no mitochondria present	In the inner membrane of mitochondria and chloroplasts

Table 1.1 Comparison of prokaryotic and eukaryotic cell organization—cont'd

Characteristic	Prokaryote	Eukaryote
Sterols in cytoplasmic membrane	Absent except in Mycoplasmataceae	Present
Plasma membrane	Phospholipid bilayer; lacks carbohydrates	Phospholipid bilayer; also contains glycolipids and glycoproteins
Cell wall, if present	Peptidoglycan in most bacteria	Cellulose, phenolic polymers, lignin (plants), chitin (fungi), other glycans (algae)
Glycocalyx	Present in many as an organized capsule or unorganized slime layer	Present; some animal cells
Cilia	Absent	Present; see description of flagella
Flagella, if present	Simple flagella; composed of polymers of flagellin; movement by rotary action at the base; spirochetes have MTs	Complex cilia or flagella; composed of MTs and polymers of tubulin with dynein connecting MTs; movement by coordinated sliding MTs
Pili and fimbriae	Present	Absent

MT, Microtubule.

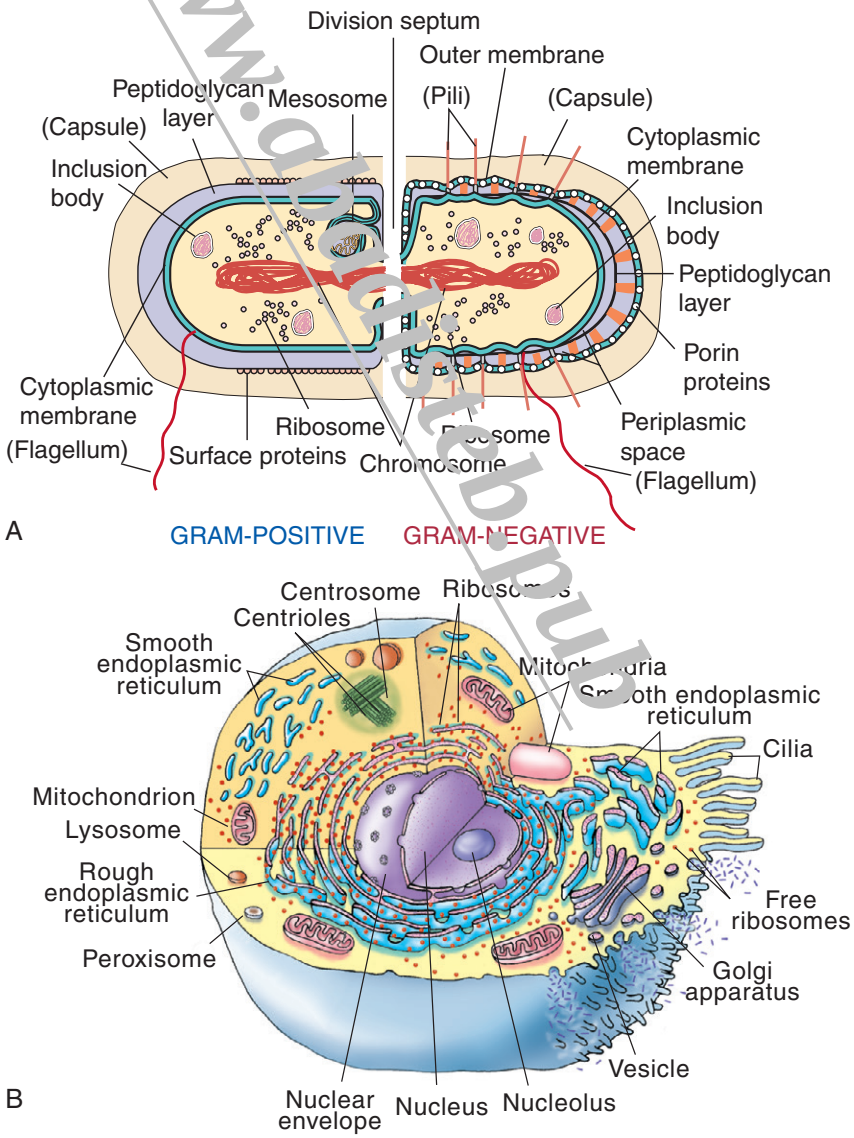

Fig. 1.1 Comparison of prokaryotic and eukaryotic cell organization and structures. A, Prokaryotic gram-positive and gram-negative bacteria. B, Structure of the generalized eukaryotic cell. (*A, From Murray, P. R., et al. (2009). Medical microbiology [6th ed]. Philadelphia: Mosby; B, from Thibodeau, G. A., & Patton, K. T. (2007). Anatomy and physiology [6th ed]. St Louis: Mosby.)*

forms), growing as yeast at human temperature (37° C) and as the filamentous form at room temperature (22° C). These fungi are called **dimorphic**. Some systemic fungal diseases in human hosts are caused by dimorphic fungi (see Chapter 27).

Viruses

Viruses are the smallest infectious particles and cannot be seen under an ordinary light microscope. Often, we can see their effects on cell lines grown in the laboratory, such as inclusions, rounding up of cells, and syncytium (fusion of host cells into multinucleated infected forms), where these characteristics become diagnostic for many viral diseases. These visible changes are called *cytopathic effect*. Viruses are acellular (not composed of cells) and are therefore neither prokaryotic nor eukaryotic. They are distinguished from living cells by the following characteristics:

- Viruses consist of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), but rarely both. Their genome can be double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), or single-stranded RNA (ssRNA).
- Viruses lack cytoplasmic membranes and are surrounded by a protein coat.
- Viruses are obligate intracellular parasites that cannot self-replicate. They require host cells for replication (increase in number does not involve mitosis, meiosis, or binary fission) and metabolism. Because they lack ribosomes and other metabolites, they "take over" host cell function using the host cell machinery to reproduce. Growth (increase in size) does not occur in viruses.

Viruses are mostly host or host cell specific. For example, human immunodeficiency virus infects T-helper lymphocytes, not muscle cells, in humans, whereas other viruses, such as the rabies virus, can infect dogs, skunks, bats, humans, and other animals. A virus that infects and possibly destroys bacterial cells is known as a **bacteriophage** (Greek *phage:* to eat). Viruses are classified and identified by their genome (DNA or RNA), host range, host disease signs and symptoms, chemical makeup, geographic distribution, the presence or absence of an envelope, their resistance to changes in pH and temperature, their antigenicity (serologic methods), how the virus replicates, and the **virion** (a complete virus outside a cell) size (see Chapter 29).

Classification/Taxonomy

Taxonomy (Greek *taxes:* arrangement; Greek *nomos:* law) is the orderly classification and grouping of organisms into **taxa** (categories). Taxonomy involves three structured, interrelated categories: classification/taxonomy, nomenclature, and identification. It is based on similarities and differences in **genotype** (genetic makeup of an organism, or combinations of forms of one or a few genes in an organism's genome) and **phenotype** (observable physical and functional features of an organism expressed by its genotype). Examples of genotypic characteristics include base sequencing of DNA or RNA and DNA base composition ratio to measure the degree of relatedness of two organisms (see later in this chapter and Chapter 11). Examples of microbial phenotypic characteristics include macroscopic (colony morphology on media) and microscopic morphology (size, shape, arrangement into groups or chains of cells), staining characteristics (gram-positive or gram-negative), nutritional requirements, physiologic and biochemical characteristics, antigenic markers, and susceptibility or resistance to antimicrobial agents or chemicals. See Chapters 7, 8, 9, 12, and 13 for more detailed information.

Taxa (plural of *taxon*), for example, the levels of classification, are the categories or subsets in taxonomy. The formal levels of bacterial classification in successively smaller taxa or subsets are domain, kingdom, division (or phylum in kingdom Animalia), class, order, family, tribe, genus, species, and subspecies. Below the subspecies level, designations such as serotype or biotype may be given to organisms that share specific minor characteristics. Protists (protozoans) of clinical importance are named similarly to animals; instead of divisions, **phyla** (plural of *phylum*) is used, but the names of the other classifications remain the same. Prokaryotes are placed in the domains Bacteria and **Archaea** (Greek: ancient, origin from the earliest cells), separate from the animals; plants and protists are placed in the domain **Eukarya**. The domains Bacteria and Archaea include unicellular prokaryotic organisms.

Clinical microbiologists traditionally emphasize placement and naming of bacterial species into three (occasionally four or five) categories: the **family** (similar to a human "clan"), a **genus** (equivalent to a human last name), and a **species** (equivalent to a human first name). The plural of genus is *genera*. For example, there are many genera in the family Staphyloccocceae. The proper word for the name of a species is an *epithet*. For example, *Staphylococcus* (genus) *aureus* (species epithet) belongs to the family Staphylococcaceae. Although order and tribe may be useful for the classification of plants and animals, these taxa are not always used for the classification of bacteria. In addition, there are usually different strains within a given species of the same species. As an example, there are many different strains of *S. aureus*. If the *S. aureus* isolated from one patient is resistant to penicillin and another *S. aureus* isolate from a different patient is susceptible to penicillin, the two isolates belong to different strains of the same species.

Nomenclature

Nomenclature provides naming assignments for each organism. The following standard rules for denoting bacterial names are used. The family name is capitalized and has an *"-aceae"* ending (e.g., Micrococcaceae). The genus name is capitalized and followed by the species epithet, which begins with a lowercase letter; both the genus and species should be italicized in print but underlined when written in script (e.g., *Staphylococcus aureus* or Staphylococcus aureus). Often the genus name is abbreviated by use of the first letter (capitalized) of the genus followed by a period and the species epithet (e.g., *S. aureus*). The genus name followed by the word *species* (e.g., *Staphylococcus* species) may be used to refer to the genus as a whole. Species are abbreviated "sp." (singular) or "spp." (plural) when the species is not specified. When bacteria are referred to as a group, their names are neither capitalized nor underlined (e.g., staphylococci).

Classification by phenotypic and genotypic characteristics

The traditional method of placing an organism into a particular genus and species is based on the similarity of all members in numerous phenotypic characteristics. In the diagnostic microbiology laboratory, this classification is accomplished by testing each bacterial culture for various metabolic or molecular characteristics and comparing the results with those listed in established tables or databases. In many rapid identification systems, a numeric taxonomy is used in which phenotypic characteristics are assigned a numeric value, and the derived number indicates the genus and species of the bacterium by consulting a database of known organisms.

Epidemiologists constantly seek means of further subdividing bacterial species to follow the spread of bacterial infections. Species are subdivided into subspecies (abbreviated "subsp.") on the basis of phenotypic differences, serovarieties (abbreviated "serovar") on the basis of serologic (antigenic) differences, or biovarieties (abbreviated "biovar") on the basis of biochemical test result differences. Phage typing (based on susceptibility to specific bacterial phages) is also used for this purpose. Current technology allows the analysis of genetic relatedness (DNA and RNA structure and homology) for taxonomic purposes. The analysis of ribosomal RNA (rRNA) gene sequencing is particularly useful for this purpose. The information obtained from these studies resulted in the reclassification of some bacteria.

Classification by cellular type: prokaryotes, eukaryotes, and archaea

Based on cell organization and function, organisms fall into one of three distinct groups: prokaryotes, eukaryotes, or archaea. Taxonomists placed all organisms into three domains that replaced some kingdoms: Bacteria, Archaea, and Eukarya. These three domains are the largest and most inclusive taxa. Each domain is divided into kingdoms on the basis of the similarities of RNA, DNA, and protein sequences. The group prokaryotes includes the domains Archaea and Bacteria (Eubacteria), whereas fungi, algae, protozoa, animals, and plants are eukaryotic in nature and are placed in the domain Eukarya.

The domain Archaea (formerly Archaeobacteria) cell type appears to be more closely related to eukaryotic cells than to prokaryotic cells and is found in microorganisms that grow under extreme environmental conditions. Archaeal cell walls lack peptidoglycan, a major reason they are placed in a domain separate from bacteria. These microbes share some common characteristics with bacteria; they too can stain gram-positive or gram-negative. Gram-positive archaea have a thick wall and stain purple. Gram-negative archaeal cells, in contrast with the typical gram-negative bacterial lipid membrane, have a layer of protein covering the cell wall and stain pink. See the "Gram Stain" section later in this chapter.

The structure of the cell envelope and enzymes of archaea allows them to survive under stressful or extreme (*extremophiles;* lovers of the extreme) conditions. Examples include **halophiles** (salt-loving cells) in Utah's Great Salt Lake, **thermophiles** (heat-loving cells) in hot springs and deep ocean vents, and the anaerobic methanogens that release swamp gas and inhabit the intestinal tracts of animals. Because archaea are not encountered in clinical microbiology, they are not discussed further in this textbook.

In general, the interior organization of eukaryotic cells is more complex than that of prokaryotic cells (see Fig. 1.1). The eukaryotic cell is usually larger and contains membrane-encased organelles ("little organs") or compartments that serve specific functions, whereas the prokaryotic cell is noncompartmentalized. Various structures are unique to prokaryotic cells (see Fig. 1.1). Differences also exist in the processes of DNA synthesis, protein synthesis, and cell wall synthesis and structure. Table 1.1 compares the major characteristics of eukaryotic and prokaryotic cells.

Pathogenic (disease-causing) **bacteria** are prokaryotic cells that infect eukaryotic hosts. Antimicrobial agents targeting unique prokaryotic structures and metabolism inhibit bacterial growth without harming eukaryotic host cells. This is one reason that pharmaceutical companies have been successful in developing effective antimicrobial agents against bacterial pathogens. However, finding drugs effective against parasites and fungi, which are eukaryotic and resemble their human hosts, and viruses, which use host cells for replication, is less successful. In addition, as a result of genetic changes, bacteria continue to acquire drug resistance.

Comparison of prokaryotic and eukaryotic cell structure

Prokaryotic cell structure

Cytoplasmic structures

Bacteria do not contain a membrane-bound nucleus. Their genome consists of a single circular chromosome. This appears as a diffuse nucleoid or chromatin body (nuclear body) that is attached to a mesosome, a saclike structure in the cell membrane.

Bacterial ribosomes, consisting of RNA and protein, are found free in the cytoplasm and attached to the cytoplasmic membrane. They are the site of protein synthesis. They are 70S in size and dissociate into two subunits: 50S and 30S (see Table 1.1). The *S* stands for *Svedberg units*, which refer to sedimentation rates (unit of time) during high-speed centrifugation. The Svedberg unit is named for Theodor Svedberg, Nobel Prize winner and inventor of the ultracentrifuge. Larger particles have higher S values. The S value is *not additive*. When the previously mentioned two subunits 50S and 30S bind together, there is a loss of surface area, and the two subunits produce a complex 70S in size. The same occurs in the eukaryotic cell, where the two subunits 60S and 40S combine to form an 80S complex.

Stained bacteria sometimes reveal the presence of cytoplasmic granules. These granules are storage deposits and may consist of polysaccharides such as glycogen, lipids such as poly β-hydroxybutyrate, or polyphosphates. These granules are sometimes referred to as *metachromatic granules* and can be visualized with the methylene blue stain.