10

Classification of Microorganisms

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The science of classification, especially the classification of living forms, is called *taxonomy* (from the Greek for orderly arrangement). The objective of taxonomy is to classify living organisms—that is, to establish the relationships between one group of organisms and another and to differentiate them. There may be as many as 100 million different living organisms, but fewer than 10% have been discovered, much less classified and identified.

Taxonomy also provides a common reference for identifying organisms already classified. For example, when a bacterium suspected of causing a specific disease is isolated from a patient, characteristics of that isolate are matched to lists of characteristics of previously classified bacteria to identify the isolate (see the box on page 282). Finally, taxonomy is a basic and necessary tool for scientists, providing a universal language of communication.

Modern taxonomy is an exciting and dynamic field. The ability to rapidly sequence DNA, even entire genomes, has led to new insights into classification and evolution. In this chapter, you will learn the various classification systems, the different criteria used for classification, and tests that are used to identify microorganisms that have already been classified. The contiribution of taxonomy in shedding new light on previously discovered organisms like the *Pneumocystis jirovecii* shown in the photograph will be discussed in this chapter.

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The Study of Phylogenetic Relationships

LEARNING OBJECTIVES

10–1 Define *taxonomy*, *taxon*, and *phylogeny*.

10–2 Discuss the limitations of a two-kingdom classification system.

10–3 Identify the contributions of Linnaeus, von Nägeli, Chatton, Whittaker, and Woese.

10–4 Discuss the advantages of the three-domain system.

10–5 List the characteristics of the Bacteria, Archaea, and Eukarya domains.

In 2001, an international project called the All Species Inventory was launched. The project's purpose is to identify and record every species of life on Earth in the next 25 years. These researchers have undertaken a challenging goal: whereas biologists have identified more than 1.7 million different organisms thus far, it is estimated that the number of living species ranges from 10 to 100 million.

Among these many and diverse organisms, however, are many similarities. For example, all organisms are composed of cells surrounded by a plasma membrane, use ATP for energy, and store their genetic information in DNA. These similarities are the result of evolution, or descent from a common ancestor. In 1859, the English naturalist Charles Darwin proposed that natural selection was responsible for the similarities as well as the differences among organisms. The differences can be attributed to the survival of organisms with traits best suited to a particular environment.

To facilitate research, scholarship, and communication, we use **taxonomy**—that is, we put organisms into categories, or **taxa** (singular: *taxon*), to show degrees of similarities among organisms. These similarities are due to relatedness—all organisms are related through evolution. **Systematics,** or **phylogeny,** is the study of the evolutionary history of organisms. The hierarchy of taxa reflects evolutionary, or *phylogenetic,* relationships.

From the time of Aristotle, living organisms were categorized in just two ways, as either plants or animals. In 1735, the Swedish botanist Carolus Linnaeus introduced a formal system of classification dividing living organisms into two kingdoms—Plantae and Animalia. He used latinized names to provide one common "language" for systematics. As the biological sciences developed, however, biologists began looking for a *natural* classification system—one that groups organisms based on ancestral relationships and allows us to see the order in life. In 1857, Carl von Nägeli, a contemporary of Pasteur, proposed that bacteria and fungi be placed in the plant kingdom. In 1866, Ernst Haeckel proposed the Kingdom Protista, to include bacteria, protozoa, algae, and fungi. Because of disagreements over the definition of protists, for the next 100 years biologists continued to follow von Nägeli's placement of bacteria and fungi in the plant kingdom. It is ironic that recent DNA sequencing places fungi closer to animals than plants. Fungi were placed in their own kingdom in 1959.

With the advent of electron microscopy, the physical differences between cells became apparent. The term *prokaryote* was introduced in 1937 by Edouard Chatton to distinguish cells having no nucleus from the nucleated cells of plants and animals. In 1961, Roger Stanier provided the current definition of *prokaryotes*: cells in which the nuclear material (nucleoplasm) is not surrounded by a nuclear membrane. In 1968, Robert G.E. Murray proposed the Kingdom Prokaryotae.

In 1969, Robert H. Whittaker founded the five-kingdom system in which prokaryotes were placed in the Kingdom Prokaryotae, or Monera, and eukaryotes comprised the other four kingdoms. The Kingdom Prokaryotae had been based on microscopic observations. Subsequently, new techniques in molecular biology revealed that there are actually two types of prokaryotic cells and one type of eukaryotic cell.

CHECK YOUR UNDERSTANDING

- Of what value is taxonomy and systematics? **10-1**
- Why shouldn't bacteria be placed in the plant kingdom? **10-2, 10-3**

The Three Domains

The discovery of three cell types was based on the observations that ribosomes are not the same in all cells (see Chapter 4, page 94). Ribosomes provide a method of comparing cells because ribosomes are present in all cells. Comparing the sequences of nucleotides in ribosomal RNA (see page 292) from different kinds of cells shows that there are three distinctly different cell groups: the eukaryotes and two different types of prokaryotes—the bacteria and the archaea.

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Clinical Case: Full-Flavor Outbreak

Monica Jackson, a 32-year-old production assistant at a Reno, Nevada, television station, has made an appointment with the nurse practitioner at her physician's office. Monica tells the nurse practitioner that she has had diarrhea, nausea, and abdominal cramping for almost 12 hours. She also feels tired and has a low-grade fever. Monica felt fine one minute, and the next she was violently ill. Monica informs the nurse practitioner that she and her good friend, who is also sick, had been at the same luncheon the day before. The nurse practitioner takes a stool sample and sends it to the hospital laboratory for analysis.

What will the laboratory do first to look for a bacterial pathogen? Read on to find out.

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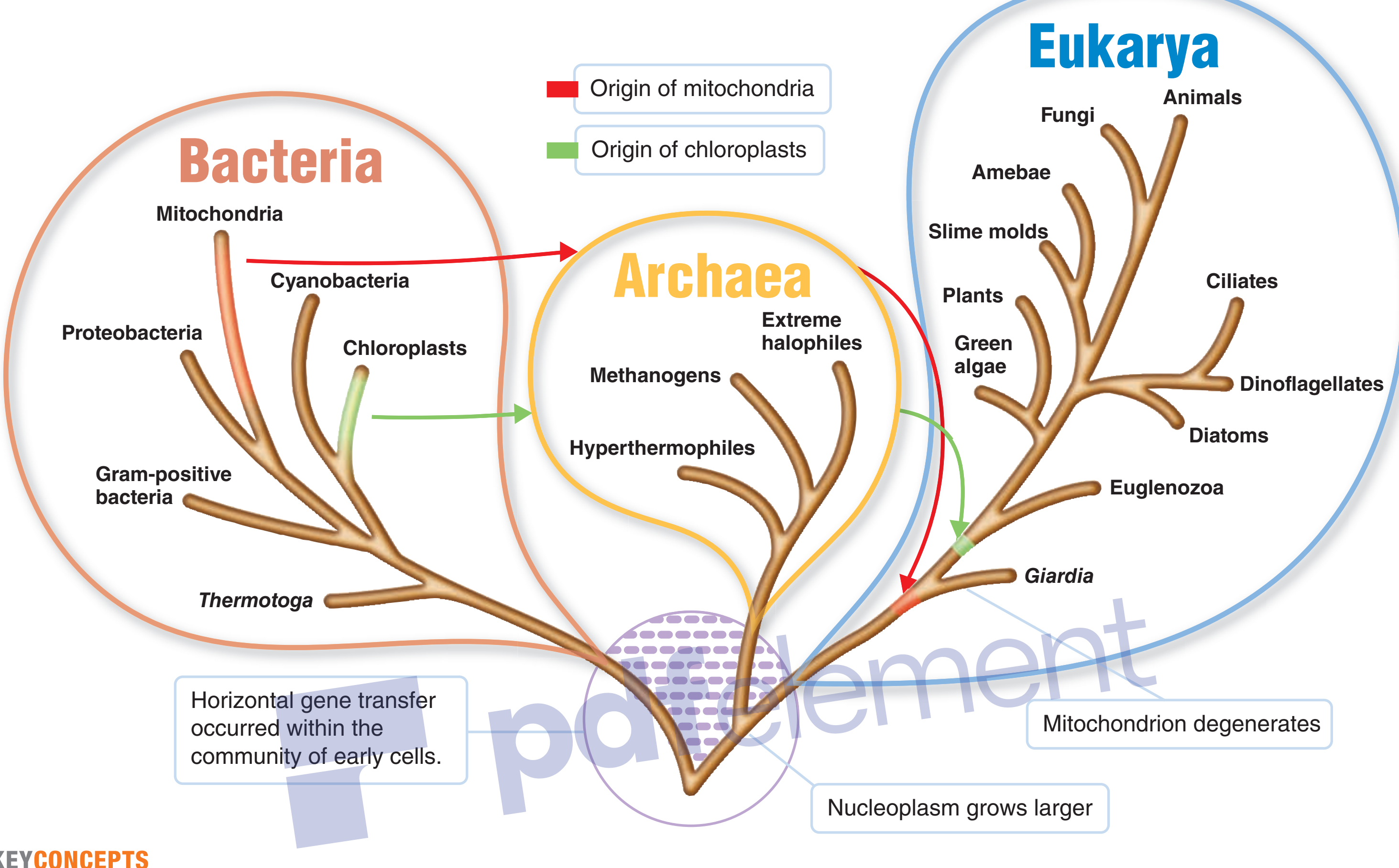
FOUNDATION FIGURE **10.1**

The Three-Domain System



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KEY CONCEPTS

- All organisms evolved from cells that formed over 3 billion years ago.
- The DNA passed on from ancestors is described as *conserved*.
- The Domain Eukarya includes the Kingdoms Fungi, Plantae, and Animalia, as well as protists. The Domains Bacteria and Archaea are prokaryotes.

In 1978, Carl R. Woese proposed elevating the three cell types to a level above kingdom, called domain. Woese believed that the archaea and the bacteria, although similar in appearance, should form their own separate domains on the evolutionary tree (**Figure 10.1**). Organisms are classified by cell type in the three domain systems. In addition to differences in rRNA, the three domains differ in membrane lipid structure, transfer RNA molecules, and sensitivity to antibiotics (**Table 10.1**).

In this widely accepted scheme, animals, plants, and fungi are kingdoms in the Domain **Eukarya.** The Domain **Bacteria** includes all of the pathogenic prokaryotes as well as many of the nonpathogenic prokaryotes found in soil and water. The photoautotrophic prokaryotes are also in this domain. The Domain **Archaea** includes prokaryotes that do not have peptidoglycan in their cell walls. They often live in extreme environments and carry out unusual metabolic processes. Archaea include three major groups:

1. The methanogens, strict anaerobes that produce methane (CH_4) from carbon dioxide and hydrogen.
2. Extreme halophiles, which require high concentrations of salt for survival.
3. Hyperthermophiles, which normally grow in extremely hot environments.

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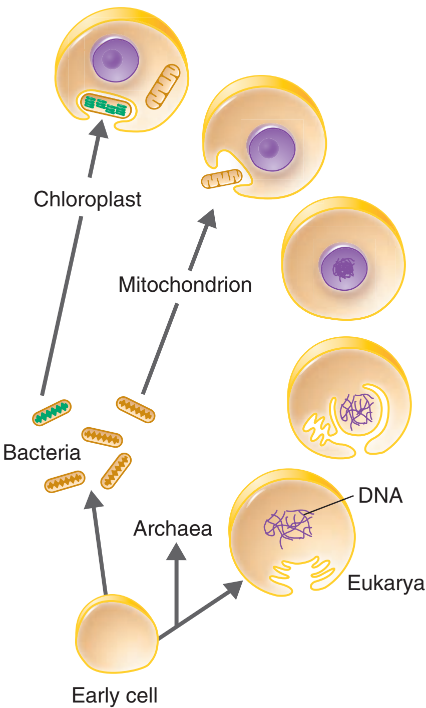

Figure 10.2 A model of the origin of eukaryotes. Invagination of the plasma membrane may have formed the nuclear envelope and endoplasmic reticulum. Similarities, including rRNA sequences, indicate that endosymbiotic prokaryotes gave rise to mitochondria and chloroplasts.

Q How many membranes make up the nuclear envelope of a eukaryotic cell?

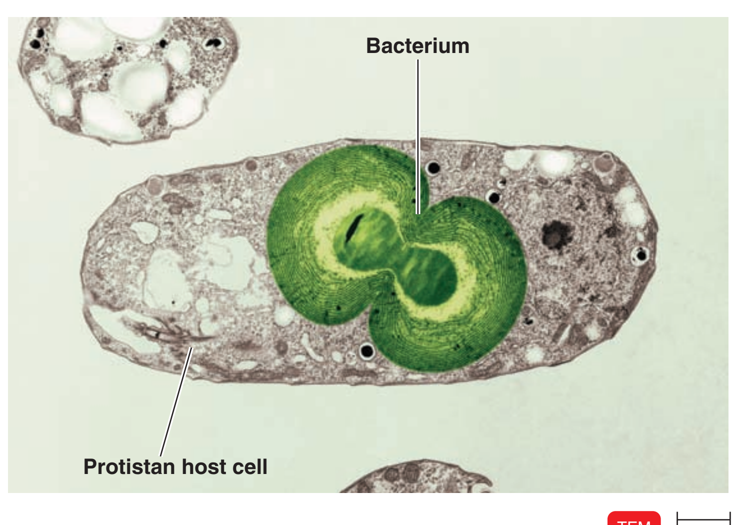

Figure 10.3 *Cyanophora paradoxa*. This organism, in which the eukaryotic host and the bacterium require each other for survival, provides a modern example of how eukaryotic cells might have evolved.

Q What features do chloroplasts, mitochondria, and bacteria have in common?

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The evolutionary relationship of the three domains is the subject of current research by biologists. Based on rRNA analysis, three cell lineages clearly emerged as cells were forming 3.5 billion years ago. That led to the Archaea, the Bacteria, and what eventually became the nucleoplasm of the eukaryotes. However, the three cell lines were not isolated; horizontal gene transfer (page 232) appears to have occurred among them. Analysis of complete genomes shows that each domain shares genes with other domains. One-quarter of the genes of the bacterium *Thermotoga* were probably acquired from an archaeon. Gene transfer also has been seen between eukaryotic hosts from their prokaryote symbionts (see the box on page 308).

The oldest known fossils are the remains of prokaryotes that lived more than 3.5 billion years ago. Eukaryotic cells evolved more recently, about 2.5 billion years ago. According to the endosymbiotic theory, eukaryotic cells evolved from prokaryotic cells living inside one another, as endosymbionts (see Chapter 4, page 106). In fact, the similarities between prokaryotic cells and eukaryotic organelles provide striking evidence for this endosymbiotic relationship (**Table 10.2**).

The original nucleoplasmic cell was prokaryotic. However, infoldings in its plasma membrane may have surrounded the nuclear region to produce a true nucleus (**Figure 10.2**). Recently, French researchers provided support for this hypothesis with their observations of a true nucleus in *Gemmata* bacteria (see Figure 11.23). Over time, the chromosome of the nucleoplasm may have acquired pieces such as transposons (page 237). In some cells, this large chromosome may have fragmented into smaller linear chromosomes. Perhaps cells with linear chromosomes had an advantage in cell division over those with a large, unwieldy circular chromosome.

That nucleoplasmic cell provided the original host in which endosymbiotic bacteria developed into organelles (see page 106). An example of a modern prokaryote living in a eukaryotic cell is shown in **Figure 10.3**. The cyanobacterium-like cell and the eukaryotic host require each other for survival.

Taxonomy provides tools for clarifying the evolution of organisms, as well as their interrelationships. New organisms are being discovered every day, and taxonomists continue to search for a natural classification system that reflects phylogenetic relationships.

A Phylogenetic Hierarchy

In a phylogenetic hierarchy, grouping organisms according to common properties implies that a group of organisms evolved from a common ancestor; each species retains some of the characteristics of the ancestor. Some of the information used to classify and determine phylogenetic relationships in higher

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TABLE 10.1 Some Characteristics of Archaea, Bacteria, and Eukarya

	Archaea	Bacteria	Eukarya
	Sulfolobus SEM 1 μm	*E. coli* SEM 1 μm	*Amoeba* SEM 5 μm
Cell Type	Prokaryotic	Prokaryotic	Eukaryotic
Cell Wall	Varies in composition; contains no peptidoglycan	Contains peptidoglycan	Varies in composition; contains carbohydrates
Membrane Lipids	Composed of branched carbon chains attached to glycerol by ether linkage	Composed of straight carbon chains attached to glycerol by ester linkage	Composed of straight carbon chains attached to glycerol by ester linkage
First Amino Acid in Protein Synthesis	Methionine	Formylmethionine	Methionine
Antibiotic Sensitivity	No	Yes	No
rRNA Loop*	Lacking	Present	Lacking
Common Arm of tRNA†	Lacking	Present	Present

*Binds to ribosomal protein; found in all bacteria.
†A sequence of bases in tRNA found in all eukaryotes and bacteria: guanine-thymine-pseudouridine-cytosine-guanine.

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TABLE 10.2 Prokaryotic Cells and Eukaryotic Organelles Compared

	Prokaryotic Cell	Eukaryotic Cell	Eukaryotic Organelles (Mitochondria and Chloroplasts)
DNA	One circular; some two circular; some linear	Linear	Circular
Histones	In archaea	Yes	No
First Amino Acid in Protein Synthesis	Formylmethionine (bacteria) Methionine (archaea)	Methionine	Formylmethionine
Ribosomes	70S	80S	70S
Growth	Binary fission	Mitosis	Binary fission

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organisms comes from fossils. Bones, shells, or stems that contain mineral matter or have left imprints in rock that was once mud are examples of fossils.

The structures of most microorganisms are not readily fossilized. Some exceptions are the following:

- A marine protist whose fossilized colonies form the White Cliffs of Dover, England.
- Stromatolites, the fossilized remains of filamentous bacteria and sediments that flourished between 0.5 and 2 billion years ago (**Figure 10.4a** and **Figure 10.4b**).
- Cyanobacteria-like fossils found in rocks in western Australia that are 3.0 to 3.5 billion years old. These are widely believed to be the oldest known fossils (**Figure 10.4c**).

Because fossil evidence is not available for most prokaryotes, their phylogeny must be based on other evidence. But in one notable exception, scientists may have isolated living bacteria and yeast 25 to 40 million years old. In 1995, the American microbiologist Raul Cano and his colleagues reported growing *Bacillus sphaericus* and other as yet unidentified microorganisms that had survived embedded in amber (fossilized plant resin) for millions of years. If confirmed, this discovery should provide more information about the evolution of microorganisms.

Similarities in genomes can be used to group organisms into taxa and to provide a timeline for the emergence of taxa. This is especially important for microorganisms that usually don't leave fossil evidence. This concept of a molecular clock based on the differences in amino acids in hemoglobin among different animals was first proposed in the 1960s. A **molecular clock** for evolution is based on nucleotide sequences in the genomes of organisms. Mutations accumulate in a genome at a constant rate. In some genes, such as the rRNA genes, there are few mutations. These are highly conserved genes. Other regions of a genome, change with no apparent effect on the organism. Comparing the number of mutations between two organisms with the expected rate of change provides an estimate of when the two diverged from a common ancestor. This technique was used to track the path of West Nile virus to the United States. (See the box on page 220)

Conclusions from rRNA sequencing and DNA hybridization studies (discussed on page 290) of selected orders and families of eukaryotes are in agreement with the fossil records. This has encouraged workers to use DNA hybridization and rRNA sequencing to gain an understanding of the evolutionary relationships among prokaryotic groups.

CHECK YOUR UNDERSTANDING

- What evidence supports classifying organisms into three domains? 10-4
- Compare archaea and bacteria; bacteria and eukarya; and archaea and eukarya. 10-5

(a) Bacterial communities form rocklike pillars called stromatolites. These began growing about 3000 years ago. 30 cm

(b) Cut section through a fossilized stomatolite that flourished 2 billion years ago. 2 cm

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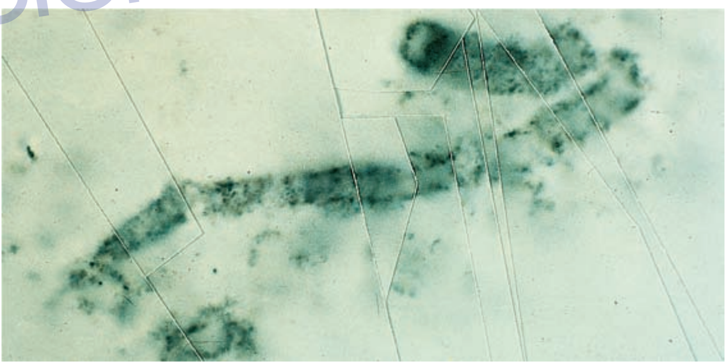

(c) Filamentous prokaryotes from the Early Precambrian (3.5 billion years ago) of western Australia.

15 μm

Figure 10.4 Fossilized prokaryotes.

What evidence is used to determine the phylogeny of prokaryotes?

Classification of Organisms

LEARNING OBJECTIVES

10-6 Explain why scientific names are used.
10-7 List the major taxa.
10-8 Differentiate *culture*, *clone*, and *strain*.
10-9 List the major characteristics used to differentiate the three kingdoms of multicellular Eukarya.
10-10 Define *protist*.
10-11 Differentiate eukaryotic, prokaryotic, and viral species.

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Living organisms are grouped according to similar characteristics (classification), and each organism is assigned a unique scientific name. The rules for classifying and naming, which are used by biologists worldwide, are discussed next.

Scientific Nomenclature

In a world inhabited by millions of living organisms, biologists must be sure they know exactly which organism is being discussed. We cannot use common names, because the same name is often used for many different organisms in different locales. For example, there are two different organisms with the common name Spanish moss, and neither one is actually a moss. Plus, local languages are used for common names. Because common names can be misleading and are in different languages, a system of scientific names, referred to as *scientific nomenclature,* was developed in the eighteenth century.

Recall from Chapter 1 (page 3) that every organism is assigned two names, or a binomial. These names are the **genus** name and **specific epithet (species),** and both names are printed underlined or italicized. The genus name is always capitalized and is always a noun. The species name is lowercase and is usually an adjective. Because this system gives two names to each organism, the system is called **binomial nomenclature.**

Let's consider some examples. Our own genus and specific epithet are *Homo sapiens* (hō′mō sā′pē-ens). The noun, or genus, means man; the adjective, or specific epithet, means wise. A mold that contaminates bread is called *Rhizopus stolonifer* (rī′zō-pụs stō′ion-i-fėr). *Rhizo-* (root) describes rootlike structures on the fungus; *stolo-* (a shoot) describes the long hyphae. Table 1.1 on page 4 contains more examples.

Binomials are used by scientists worldwide, regardless of their native language, which enables them to share knowledge efficiently and accurately. Several scientific entities are responsible for establishing rules governing the naming of organisms. Rules for assigning names for protozoa and parasitic worms are published in the *International Code of Zoological Nomenclature.* Rules for assigning names for fungi and algae are published in the *International Code of Botanical Nomenclature.* Rules for naming newly classified prokaryotes and for assigning prokaryotes to taxa are established by the International Committee on Systematics of Prokaryotes and are published in the *Bacteriological Code.* Descriptions of prokaryotes and evidence for their classifications are published in the *International Journal of Systematic and Evolutionary Microbiology* before being incorporated into a reference called *Bergey's Manual.* According to the *Bacteriological Code,* scientific names are to be taken from Latin (a genus name can be taken from Greek) or latinized by the addition of the appropriate suffix. Suffixes for order and family are *-ales* and *-aceae,* respectively.

As new laboratory techniques make more detailed characterizations of microbes possible, two genera may be reclassified as a single genus, or a genus may be divided into two or more genera. For example, the genera "Diplococcus" and *Streptococcus* were combined in 1974; the only diplococcal species is now called *Streptococcus pneumoniae.* In 1984, DNA hybridization studies indicated that "Streptococcus faecalis" and "Streptococcus faecium" were only distantly related to the other streptococcal species; consequently, a new genus called *Enterococcus* was created, and these species were renamed *E. faecalis* and *E. faecium* (fē′sē-um).

In 2001, based on DNA-DNA hybridization and rRNA studies, some species of *Chlamydia* were moved to a new genus, *Chlamydophila*, based on rRNA analysis (see page 292). Making the transition to a new name can be confusing, so the old name is often written in parentheses. For example, a physician looking for information on the cause of a patient's pneumonia-like symptoms (melioidosis) would find the bacterial name *Burkholderia (Pseudomonas) pseudomallei* (bėrk′hōld-ér-ē-ä sū-dō-mal′le-ē).

Obtaining the name of the organism is important in determining what treatment to use; antifungal drugs will not work against bacteria, and antibacterial drugs will not work against viruses.

The Taxonomic Hierarchy

All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy. Linnaeus developed this hierarchy for his classification of plants and animals. A **eukaryotic species** is a group of closely related organisms that breed among themselves. (Bacterial species will be discussed shortly.) A genus consists of species that differ from each other in certain ways but are related by descent. For example, *Quercus* (kwer′kus), the genus name for oak, consists of all types of oak trees (white oak, red oak, bur oak, velvet oak, and so on). Even though each species of oak differs from every other species, they are all related genetically. Just as a number of species make up a genus, related genera make up a **family.** A group of similar families constitutes an **order,** and a group of similar orders makes up a **class.** Related classes, in turn, make up a **phylum.** Thus, a particular organism (or species) has a genus name and specific epithet and belongs to a family, order, class, and phylum.

All phyla that are related to each other make up a **kingdom,** and related kingdoms are grouped into a **domain** (**Figure 10.5**).

CHECK YOUR UNDERSTANDING

- Using *Escherichia coli* and *Entamoeba coli* as examples, explain why the genus name must always be written out for the first use. Why is binomial nomenclature preferable to the use of common names? **10-6**
- Find the gram-positive bacteria *Staphylococcus* in Appendix F. To which bacteria is this genus more closely related: *Bacillus* or *Streptococcus?* **10-7**

Classification of Prokaryotes

The taxonomic classification scheme for prokaryotes is found in *Bergey's Manual of Systematic Bacteriology,* 2nd edition (see

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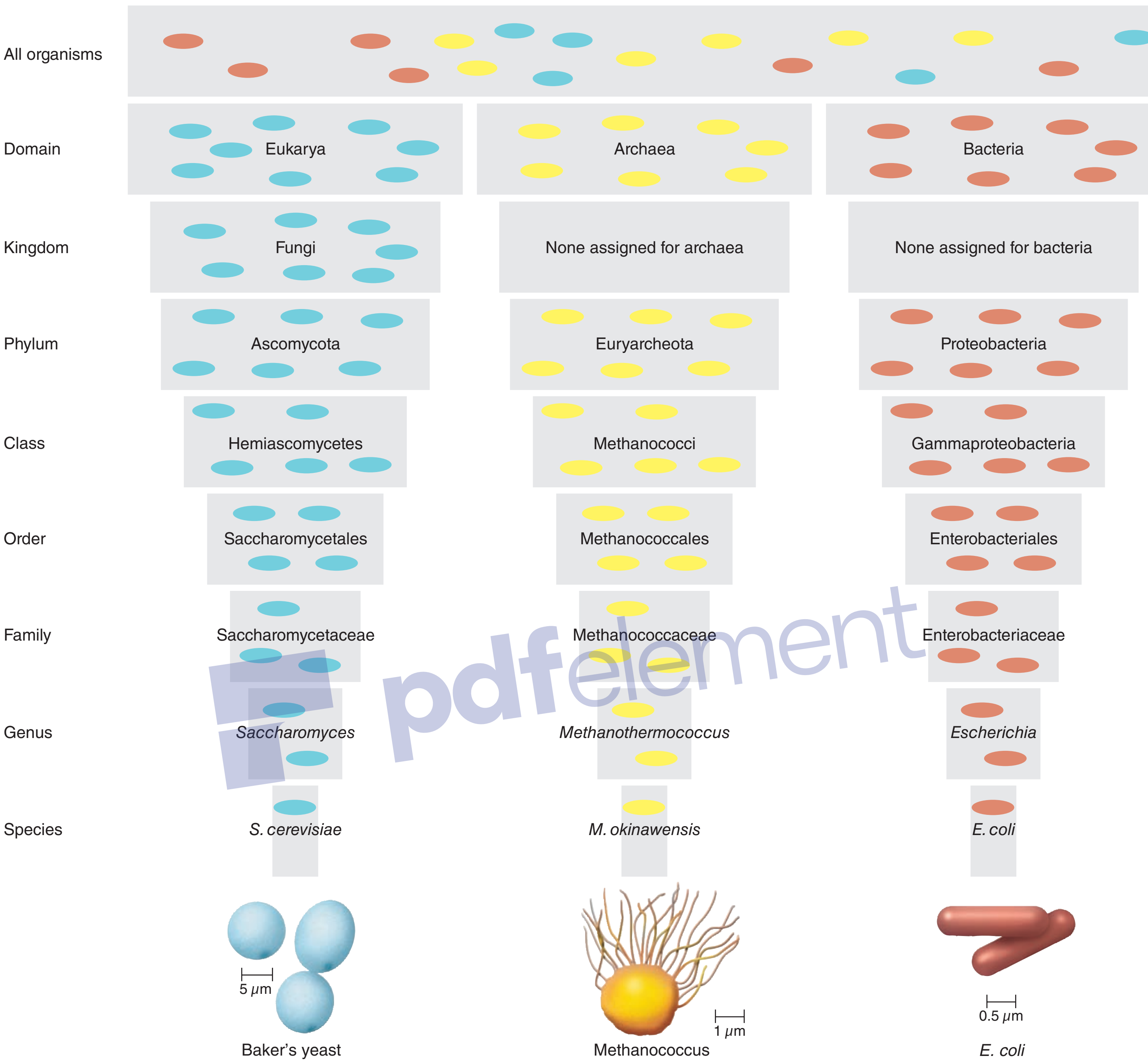

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Figure 10.5 The taxonomic hierarchy. Organisms are grouped according to relatedness. Species that are closely related are grouped into a genus. For example, the baker's yeast belongs to the genus that includes sourdough yeast (*Saccharomyces exiguus*). Related genera, such as *Saccharomyces* and *Candida*, are placed in a family, and so on. Each group is more comprehensive. The domain Eukarya includes all organisms with eukaryotic cells.

Q What is the biological definition of *family*?

Appendix F). In *Bergey's Manual,* prokaryotes are divided into two domains: Bacteria and Archaea. Each domain is divided into phyla. Remember, the classification is based on similarities in nucleotide sequences in rRNA. Classes are divided into orders; orders, into families; families, into genera; and genera, into species.

A prokaryotic species is defined somewhat differently from a eukaryotic species, which is a group of closely related organisms

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BACTERIA
Gram-positive bacteria
High G + C
Low G + C
Cyanobacteria
Proteobacteria
Chlamydias
Spirochetes
Green nonsulfur bacteria
Green sulfur bacteria
Thermotoga
Bacteroides
ARCHAEA
Methanogens
Extreme halophiles
Hyperthermophiles

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Figure 10.6 Phylogenetic relationships of prokaryotes. Arrows indicate major lines of descent of bacterial groups. Selected phyla are indicated by the white boxes.

Members of which phylum can be identified by Gram staining?

that can interbreed. Unlike reproduction in eukaryotic organisms, cell division in bacteria is not directly tied to sexual conjugation, which is infrequent and does not always need to be species-specific. A **prokaryotic species,** therefore, is defined simply as a population of cells with similar characteristics. (The types of characteristics will be discussed later in this chapter.) The members of a bacterial species are essentially indistinguishable from each other but are distinguishable from members of other species, usually on the basis of several features. As you know, bacteria grown at a given time in media are called a culture. A pure culture is often a **clone,** that is, a population of cells derived from a single parent cell. All cells in the clone should be identical. However, in some cases, pure cultures of the same species are not identical in all ways. Each such group is called a **strain.** Strains are identified by numbers, letters, or names that follow the specific epithet.

Bergey's Manual provides a reference for identifying bacteria in the laboratory, as well as a classification scheme for bacteria. One scheme for the evolutionary relationships of bacteria is shown in Figure 10.6. Characteristics used to classify and identify bacteria are discussed in Chapter 11.

Classification of Eukaryotes

Some kingdoms in the domain Eukarya are shown in Figure 10.1.

In 1969, simple eukaryotic organisms, mostly unicellular, were grouped as the Kingdom **Protista,** a catchall kingdom for a variety of organisms. Historically, eukaryotic organisms that didn't fit into other kingdoms were placed in the Protista. Approximately 200,000 species of protistans have been identified thus far, and these organisms are nutritionally quite diverse—from photosynthetic to obligate intracellular parasite. Ribosomal RNA sequencing is making it possible to divide protists into groups based on their descent from common ancestors. Consequently, for the time being, the organisms once classified as protists are being divided into **clades,** that is, genetically related groups. For convenience, we will continue to use the term *protist* to refer to unicellular eukaryotes and their close relatives. These organisms will be discussed in Chapter 12.

Fungi, plants, and animals make up the three kingdoms of more complex eukaryotic organisms, most of which are multicellular.

The Kingdom **Fungi** includes the unicellular yeasts, multicellular molds, and macroscopic species such as mushrooms.

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To obtain raw materials for vital functions, a fungus absorbs dissolved organic matter through its plasma membrane. The cells of a multicellular fungus are commonly joined to form thin tubes called *hyphae*. The hyphae are usually divided into multinucleated units by cross-walls that have holes, so that cytoplasm can flow between the cell-like units. Fungi develop from spores or from fragments of hyphae. (See Figure 12.2, page 332.)

The Kingdom **Plantae** (plants) includes some algae and all mosses, ferns, conifers, and flowering plants. All members of this kingdom are multicellular. To obtain energy, a plant uses photosynthesis, the process that converts carbon dioxide and water into organic molecules used by the cell.

The kingdom of multicellular organisms called **Animalia** (animals) includes sponges, various worms, insects, and animals with backbones (vertebrates). Animals obtain nutrients and energy by ingesting organic matter through a mouth of some kind.

Classification of Viruses

Viruses are not classified as part of any of the three domains. Viruses are not composed of cells, and they use the anabolic machinery within living host cells to multiply. A viral genome can direct biosynthesis inside a host cell, and some viral genomes can become incorporated into the host genome. The ecological niche of a virus is its specific host cell, so viruses may be more closely related to their hosts than to other viruses. The International Committee on Taxonomy of Viruses defines a **viral species** as a population of viruses with similar characteristics (including morphology, genes, and enzymes) that occupies a particular ecological niche.

Viruses are obligatory intracellular parasites. Viral genes carried in the genomes of other organisms provide a record of viral evolution. Recent analysis shows that bornavirus genes integrated into mammals, including humans, at least 40 million years ago. There are three hypotheses on the origin of viruses: (1) They arose from independently replicating strands of nucleic acids (such as plasmids). (2) They developed from degenerative cells that, through many generations, gradually lost the ability to survive independently but could survive when associated with another cell. (3) They coevolved with host cells. For example, it has been hypothesized that the bacterial cell wall provided a selection advantage to avoid getting infected. Then mutated viruses that could penetrate the cell wall would be selected. Viruses will be discussed in Chapter 13.

CHECK YOUR UNDERSTANDING

- ✓ Use the terms *species*, *culture*, *clone*, and strain in one sentence to describe growing methicillin-resistant *Staphylococcus aureus* (MRSA). **10-8**
- ✓ Assume you discovered a new organism: it is multicellular, is nucleated, is heterotrophic, and has cell walls. To what kingdom does it belong? **10-9**
- ✓ Write your own definition of *protist*. **10-10**
- ✓ Why wouldn't the definition of a viral species work for a bacterial species? **10-11**

Methods of Classifying and Identifying Microorganisms

LEARNING OBJECTIVES

10–12 Compare and contrast classification and identification.

10–13 Explain the purpose of *Bergey's Manual*.

10–14 Describe how staining and biochemical tests are used to identify bacteria.

10–15 Differentiate Western blotting from Southern blotting.

10–16 Explain how serological tests and phage typing can be used to identify an unknown bacterium.

10–17 Describe how a newly discovered microbe can be classified by DNA base composition, DNA fingerprinting, and PCR.

10–18 Describe how microorganisms can be identified by nucleic acid hybridization, Southern blotting, DNA chips, ribotyping, and FISH.

10–19 Differentiate a dichotomous key from a cladogram.

A classification scheme provides a list of characteristics and a means for comparison to aid in the identification of an organism. Once an organism is identified, it can be placed into a previously devised classification scheme. Microorganisms are *identified* for practical purposes—for example, to determine an appropriate treatment for an infection. They are not necessarily identified by the same techniques by which they are *classified*. Most identification procedures are easily performed in a laboratory and use as few procedures or tests as possible. Protozoa, parasitic worms, and fungi can usually be identified microscopically. Most prokaryotic organisms do not have distinguishing morphological features or even much variation in size and shape. Consequently, microbiologists have developed a variety of methods to test metabolic reactions and other characteristics to identify prokaryotes.

Bergey's Manual of Determinative Bacteriology has been a widely used reference since the first edition was published in 1923. The American bacteriologist David Bergey was chairman of the group who compiled information on the known bacteria from articles published in scientific journals. *Bergey's Manual of Determinative Bacteriology* (9th ed., 1994) does not classify bacteria according to evolutionary relatedness but instead provides identification (determinative) schemes based on such criteria as cell wall composition, morphology, differential staining, oxygen requirements, and biochemical testing.* The majority of Bacteria and Archaea have not been cultured, and scientists estimate that only 1% of these microbes have been discovered.

*Both Bergey's *Manual of Systematic Bacteriology* (see page 278) and *Bergey's Manual of Determinative Bacteriology* are referred to simply as *Bergey's Manual;* the complete titles are used when the information under discussion is found in one but not the other, for example, an identification table.

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Mass Deaths of Marine Mammals Spur Veterinary Microbiology

Over the past decade, thousands of marine mammals have died unexpectedly all over the world. These deaths occur in outbreaks of a dozen to thousands of mammals, and microbiologists try to determine the cause in each outbreak. The 2010 deaths of over 100 dolphins in the northern Gulf of Mexico is being investigated. These deaths occurred before the *Deepwater Horizon* well blowout in April 2010. Toxoplasmosis has been killing California sea otters in increasing numbers. The current decline in the Southern sea otter population is the result of a 40% mortality rate due to a variety of infectious bacterial diseases. These mortality figures raise concerns that entire populations of marine mammals may ultimately be destroyed.

In 2009, eight dolphin deaths in Australia were attributed to opportunistic infections. Large numbers of opportunistic pathogens, including 55 species of *Vibrio*, have also been found in dolphins. These bacteria are a part of a dolphin's normal microbiota and the biota of coastal waters. They can cause disease only if the animals' immune system, their normal defense against infection, has been weakened. The deaths of lagoon dolphins and sea otters may be due to contaminants in coastal freshwater runoff.

Phocid distemper virus in seals and cetacean morbillivirus (CM) were responsible for the deaths of 20,000 marine mammals in European waters and for recurring mortality episodes in bottlenose dolphins along the Atlantic coast of the United States. Evidence suggests that pilot whales may be responsible for transferring CM virus to other species across wide expanses of ocean.

Information Is Scarce

Such questions are the concern of veterinary microbiology, which until recently has been a neglected branch of medical microbiology. Although the diseases of such animals as cattle, chickens, and mink have been studied, partly because of their availability to researchers, the microbiology of wild animals, especially marine mammals, is a relatively newly emerging field. Gathering samples from animals that live in the open ocean and performing bacteriological analyses on them are very difficult. Currently, the animals being studied are those that have been stranded (**Figure A**) and those that come onto the shore to breed, such as the northern fur sea lion.

Microbiologists are identifying bacteria in marine mammals by using conventional test batteries (**Figure B**) and genomic data of known species. New species of bacteria are being found in marine mammals using the FISH technique (see page 292).

Veterinary microbiologists hope that increased study of the microbiology of wild animals, including marine mammals, will not only promote improved wildlife management but also provide models for the study of human diseases.

Figure A Marine mammal researchers examine a Pacific bottlenosed dolphin.

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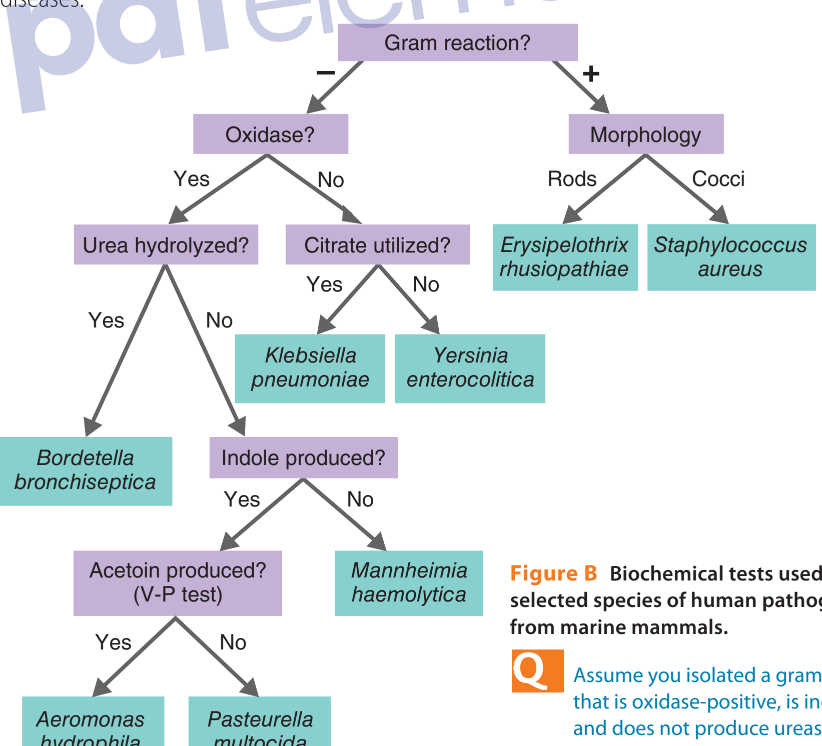

Figure B Biochemical tests used to identify selected species of human pathogens isolated from marine mammals.

Q Assume you isolated a gram-negative rod that is oxidase-positive, is indole-negative, and does not produce urease or acetoin. What is the bacterium?

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MICROBIOLOGY REQUISITION

Lab: Date, time received:	Date: Physician name:	Time: Collected by:	Slip prepared by: Patient ID#:

DO NOT WRITE BELOW THIS LINE

USE SEPARATE SLIP FOR EACH REQUEST

GRAM STAIN REPORT

- ☐ GRAM POS. COCCI, GROUPS
- ☐ GRAM POS. COCCI, PAIRS/CHAIN
- ☐ GRAM POS. RODS
- ☒ GRAM NEG. COCCI
- ☐ GRAM NEG. RODS
- ☐ GRAM NEG. COCCOBACILLI
- ☐ YEAST
- ☐ OTHER

- ☐ NO GROWTH
- ☐ NO GROWTH IN ___DAYS
- ☐ MIXED MICROBIOTA
- ☐ SPECIMEN IMPROPERLY COLLECTED OR TRANSPORTED
- ☐ ___DIFFERENT TYPES OF ORGANISMS
- ☐ NEGATIVE FOR *SALMONELLA, SHIGELLA,* AND *CAMPYLOBACTER*
- ☐ NO OVA, CYSTS, OR PARASITES SEEN
- ☒ OXIDASE-POSITIVE GRAM-NEGATIVE DIPLOCOCCI
- ☐ PRESUMPTIVE BETA STREP GROUP A BY BACITRACIN

SOURCE OF SPECIMEN

- ☐ BLOOD
- ☐ CEREBROSPINAL FLUID
- ☐ FLUID (Specify Source) ________
- ☐ THROAT
- ☐ SPUTUM, expectorated
- ☐ OTHER Respiratory (Describe) ________
- ☐ URINE, Clean Catch Midstream
- ☐ URINE, Indwelling Catheter
- ☐ URINE, Straight Catheter
- ☐ URINE, Entire First Morning
- ☐ URINE, Other (Describe) ________
- ☐ STOOL
- ☑ GU (Specify Source) ____ *vag.*
- ☐ ABSCESS (Specify Source) ________
- ☐ TISSUE (Specify Source) ________
- ☐ ULCER (Specify Source) ________
- ☐ WOUND (Specify Source) ________
- ☐ STERILIZER TEST

TEST(S) REQUESTED

Bacterial

- ☐ **Routine culture;** Gram stain, anaerobic culture, susceptibility testing. Throats done for Gp A Strep.
- ☐ *Legionella* culture
- ☐ *Bartonella*
- ☐ Blood Culture

Other Non-Routine Cultures

- ☐ *E. coli* O157:H7
- ☐ *Vibrio*
- ☐ *Yersinia*
- ☑ *H. ducreyi*
- ☐ *B. pertussis*
- ☐ Other ________

Screening Cultures

- ☑ Gonococci
- ☐ Group B Strep
- ☐ Group A Strep
- ☐ Other ________

☐ **ACID-FAST BACILLI**

☐ **FUNGAL**

VIRAL

- ☐ Routine culture
- ☐ Herpes simplex
- ☐ Direct FA for ________

PARASITOLOGY

- ☐ Exam for intestinal ova and parasites
- ☐ *Giardia* immunoassay
- ☐ *Cryptosporidium*
- ☐ Pinworm prep
- ☐ Blood parasites
- ☐ Filaria concentration
- ☐ *Trichomonas*
- ☐ Other ________

TOXIN ASSAY

- ☐ *Clostridium difficile*

DIRECT (Antigen Detection)

- ☐ Cryptococcal antigen-CSF only
- ☐ Bacterial antigens (Specify) ________

SPECIAL

- ☑ Antimicrobial tests (MIC)

Filled out by one person | Filled out by different person

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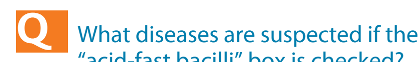
Figure 10.7 A clinical microbiology lab report form. In health care, morphology and differential staining are important in determining the proper treatment for microbial diseases. A clinician completes the form to identify the sample and specific tests. In this case, a genitourinary sample will be examined for sexually transmitted infections. The red notations are the lab technician's report of the Gram stain and culture results. [Minimal inhibitory concentration (MIC) of antibiotics will be discussed in Chapter 20, page 578]


What diseases are suspected if the "acid-fast bacilli" box is checked?

Medical microbiology (the branch of microbiology dealing with human pathogens) has dominated the interest in microbes, and this interest is reflected in many identification schemes. However, to put the pathogenic properties of bacteria in perspective, of the more than 2600 species listed in the *Approved Lists of Bacterial Names,* fewer than 10% are human pathogens.

We next discuss several criteria and methods for the classification and routine identification of microorganisms. In addition to properties of the organism itself, the source and habitat of a bacterial isolate are considered as part of the identification processes. In clinical microbiology, a physician will swab a patient's pus or tissue surface. The swab is inserted into a tube of transport medium. **Transport media** are usually not nutritive and are designed to prolong viability of fastidious pathogens. The physician will note the type of specimen and test(s) requested on a lab requisition form (Figure 10.7). The information returned by the lab technician will help the physician begin treatment (see the box in Chapter 5, page 142).

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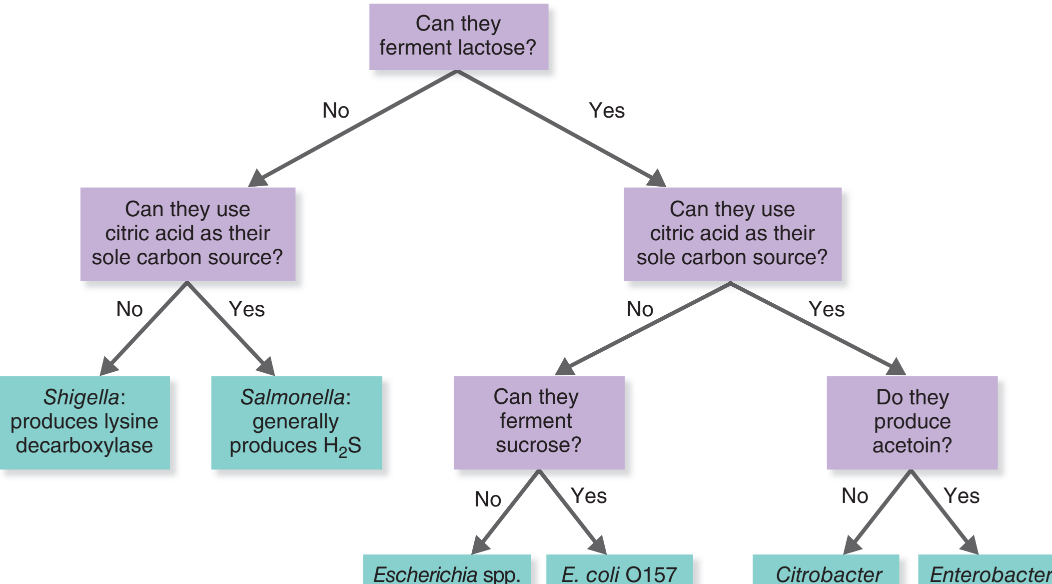

Figure 10.8 The use of metabolic characteristics to identify selected genera of enteric bacteria.

Assume you have a gram-negative bacterium that produces acid from lactose and cannot use citric acid as its sole carbon source. What is the bacterium?

Morphological Characteristics

Morphological (structural) characteristics have helped taxonomists classify organisms for 200 years. Higher organisms are frequently classified according to observed anatomical detail. But many microorganisms look too similar to be classified by their structures. Through a microscope, organisms that might differ in metabolic or physiological properties may look alike. Literally hundreds of bacterial species are small rods or small cocci.

Larger size and the presence of intracellular structures do not always mean easy classification, however. *Pneumocystis* (nü-mō-sis′ tis) pneumonia is the most common opportunistic infection in AIDS and other immunocompromised patients. Until the AIDS epidemic, the causative agent of this infection, *P. jirovecii* (ye-rō′ vet-zē-ē) [formerly "P. carinii" (kär-i′ nē-ē)] was rarely seen in humans. *Pneumocystis* lacks structures that can be easily used for identification (see Figure 24.20, page 705), and its taxonomic position has been uncertain since its discovery in 1909 by Carlos Chagas in mice. It was originally classified as a protozoan; however, in 1988 rRNA sequencing showed that *Pneumocystis* is actually a member of the Kingdom Fungi. New treatments are being investigated as researchers take into account this organism's relatedness to fungi.

Cell morphology tells us little about phylogenetic relationships. However, morphological characteristics are still useful in identifying bacteria. For example, differences in such structures as endospores or flagella can be helpful.

Differential Staining

Recall from Chapter 3 that one of the first steps in identifying bacteria is differential staining. Most bacteria are either gram-positive or gram-negative. Other differential stains, such as the acid-fast stain, can be useful for a more limited group of microorganisms. Recall that these stains are based on the chemical composition of cell walls and therefore are not useful in identifying either the wall-less bacteria or the archaea with unusual walls. Microscopic examination of a Gram stain or an acid-fast stain is used to obtain information quickly in the clinical environment.

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Biochemical Tests

Enzymatic activities are widely used to differentiate bacteria. Even closely related bacteria can usually be separated into distinct species by subjecting them to biochemical tests, such as one to determine their ability to ferment an assortment of selected carbohydrates. For one example of the use of biochemical tests to identify bacteria (in this instance, in marine mammals), see the box on page 282. Moreover, biochemical tests can provide insight into a species' niche in the ecosystem. For example, a bacterium that can fix nitrogen gas or oxidize elemental sulfur will provide important nutrients for plants and animals. This will be discussed in Chapter 27.

Enteric, gram-negative bacteria are a large heterogeneous group of microbes whose natural habitat is the intestinal tract of humans and other animals. This family contains several pathogens that cause diarrheal illness. A number of tests have been developed so that technicians can quickly identify the pathogens, a clinician can then provide appropriate treatment, and epidemiologists can locate the source of an illness. All members of the family Enterobacteriaceae are oxidase-negative. Among the enteric bacteria are members of the genera *Escherichia, Enterobacter, Shigella, Citrobacter,* and *Salmonella*. *Escherichia, Enterobacter,* and *Citrobacter,* which ferment lactose to produce acid and gas, can be distinguished from *Salmonella* and *Shigella,* which do not. Further biochemical testing, as represented in **Figure 10.8**, can differentiate among the genera.

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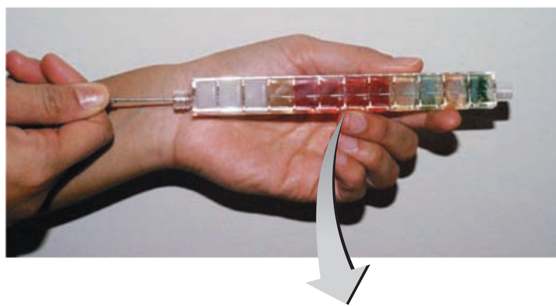
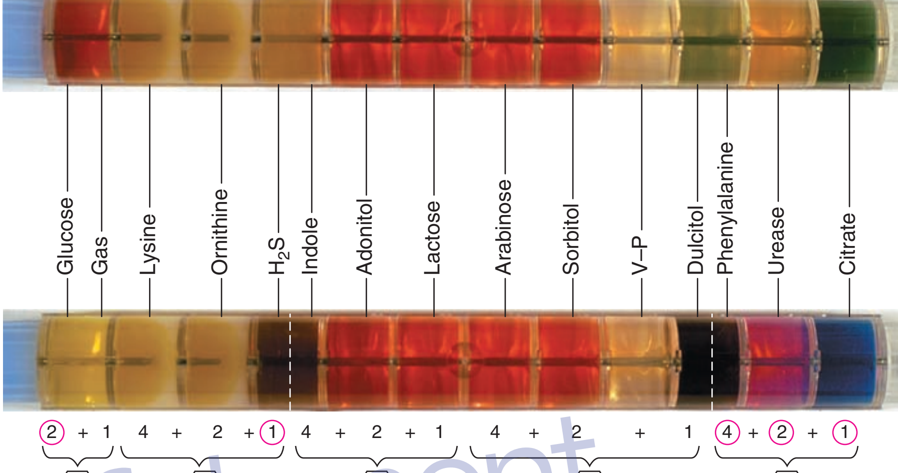
1 One tube containing media for 15 biochemical tests is inoculated with an unknown enteric bacterium.



2 After incubation, the tube is observed for results.



3 The value for each positive test is circled, and the numbers from each group of tests are added to give the ID value.

4 Comparing the resultant ID value with a computerized listing shows that the organism in the tube is *Proteus mirabilis*.

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ID Value	Organism	Atypical Test Results	Confirmatory Test
21006	*Proteus mirabilis*	Ornithine⁻	Sucrose
21007	*Proteus mirabilis*	Ornithine⁻	
21020	*Salmonella choleraesuis*	Lysine⁻	

Figure 10.9 One type of rapid identification method for bacteria: Enterotube II from Becton Dickinson. This example shows results for a typical strain of *P. mirabilis;* however, other strains may produce different test results, which are listed in the Atypical Test Results column. The V-P test is used to confirm an identification.

Q How can one species have two different ID values?

The time needed to identify bacteria can be reduced considerably by the use of selective and differential media or by rapid identification methods. Recall from Chapter 6 (page 165) that selective media contain ingredients that suppress the growth of competing organisms and encourage the growth of desired ones, and that differential media allow the desired organism to form a colony that is somehow distinctive.

Bergey's Manual does not evaluate the relative importance of each biochemical test and does not always describe strains. In diagnosing an infection, clinicians must identify a particular species and even a particular strain to proceed with proper treatment. To this end, specific series of biochemical tests have been developed for fast identification in hospital laboratories. Rapid test systems have been developed for yeasts and other fungi, as well as bacteria.

Rapid identification methods are manufactured for groups of medically important bacteria, such as the enterics. Such tools are designed to perform several biochemical tests simultaneously and can identify bacteria within 4 to 24 hours. This is sometimes called **numerical identification** because the results of each test are assigned a number. In the simplest form, a positive test would be assigned a value of 1, and a negative is assigned a value of 0. In most commercial testing kits, test results are assigned numbers ranging from 1 to 4 that are based on the relative reliability and importance of each test, and the resulting total is compared to a database of known organisms.

In the example shown in **Figure 10.9**, an unknown enteric bacterium is inoculated into a tube designed to perform 15 biochemical tests. After incubation, results in each compartment

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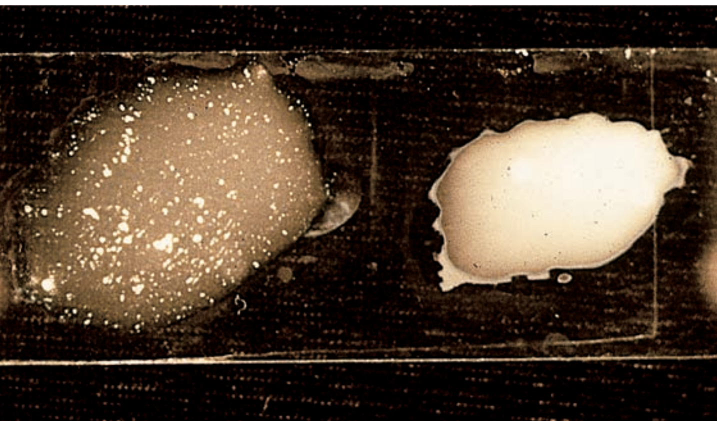

Figure 10.10 A slide agglutination test. (**a**) In a positive test, the grainy appearance is due to the clumping (agglutination) of the bacteria. (**b**) In a negative test, the bacteria are still evenly distributed in the saline and antiserum.

are recorded. Notice that each test is assigned a value; the number derived from scoring all the tests is called the ID value. Fermentation of glucose is important, and a positive reaction is valued at 2, compared with the production of acetoin (V–P test, or the Voges–Proskauer test), which has no value.

A computerized interpretation of the simultaneous test results is essential and is provided by the manufacturer. A limitation of biochemical testing is that mutations and plasmid acquisition can result in strains with different characteristics. Unless a large number of tests is used, an organism could be incorrectly identified.

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Clinical Case

The laboratory cannot just Gram stain a stool sample to look for a bacterial pathogen. The large number of gram-negative rods would be indistinguishable in a Gram stain made directly from feces. The stool sample should be cultured on selective and differential media to distinguish among bacteria in the stool. Monica's stool sample is cultured on bismuth sulfite agar. Black colonies are present on the agar after 24 hours.

Can gram-positive bacteria grow on this medium? Refer to Chapter 6 if you need a hint.

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Serology

Serology is the science that studies serum and immune responses that are evident in serum (see Chapter 18). Microorganisms are antigenic; that is, microorganisms that enter an animal's body stimulate it to form antibodies. Antibodies are proteins that circulate in the blood and combine in a highly specific way with the bacteria that caused their production. For example, the immune system of a rabbit injected with killed typhoid bacteria (antigens) responds by producing antibodies against typhoid bacteria. Solutions of such antibodies used in the identification of many medically important microorganisms are commercially available; such a solution is called an **antiserum** (plural: *antisera*). If an unknown bacterium is isolated from a patient, it can be tested against known antisera and often identified quickly.

In a procedure called a **slide agglutination test,** samples of an unknown bacterium are placed in a drop of saline on each of several slides. Then a different known antiserum is added to each sample. The bacteria agglutinate (clump) when mixed with antibodies that were produced in response to that species or strain of bacterium; a positive test is indicated by the presence of agglutination. Positive and negative slide agglutination tests are shown in Figure 10.10.

Serological testing can differentiate not only among microbial species, but also among strains within species. Strains with different antigens are called **serotypes, serovars,** or **biovars.** See the discussion of *Escherichia* and *Salmonella* serovars on page 310. As mentioned in Chapter 1, Rebecca Lancefield was able to classify serotypes of streptococci by studying serological reactions. She found that the different antigens in the cell walls of various serotypes of streptococci stimulate the formation of different antibodies. In contrast, because closely related bacteria also produce some of the same antigens, serological testing can be used to screen bacterial isolates for possible similarities. If an antiserum reacts with proteins from different bacterial species or strains, these bacteria can be tested further for relatedness.

Serological testing was used to determine whether the increase in number of cases of necrotizing fasciitis in the United States and England since 1987 was due to a common source of the infections. No common source was located, but there has been an increase in two serotypes of *Streptococcus pyogenes* that have been dubbed the "flesh-eating" bacteria.

A test called the **enzyme-linked immunosorbent assay (ELISA)** is widely used because it is fast and can be read by a computer scanner (Figure 10.11; see also Figure 18.14, page 523). In a direct ELISA, known antibodies are placed in (and adhere to) the wells of a microplate, and an unknown type of bacterium is added to each well. A reaction between the known antibodies and the bacteria provides identification of the bacteria. An ELISA is used in AIDS testing to detect the presence of antibodies against human immunodeficiency virus (HIV), the virus that causes AIDS (see Figure 19.13, page 546).

Another serological test, **Western blotting,** is also used to identify antibodies in a patient's serum (Figure 10.12). HIV infection

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Figure 10.11 An ELISA test.

Q What are the similarities between the slide agglutination test and the ELISA test?

(a) A technician uses a micropipette to add samples to a microplate for an ELISA.

(b) ELISA results are then read by the computer scanner.

is confirmed by Western blotting, and Lyme disease, caused by *Borrelia burgdorferi,* is often diagnosed by the Western blot.

1. Proteins from a known bacterium or virus are separated by an electric current in electrophoresis.
2. The proteins are then transferred to a filter by blotting.
3. Patient's serum is washed over the filter. If the patient has antibodies to one of the proteins in the filter (in this case, *Borrelia* proteins), the antibodies and protein will combine. Anti-human serum linked to an enzyme is then washed over the filter.
4. This will be made visible as a colored band on the filter after addition of the enzyme's substrate.

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Clinical Case

Bismuth sulfite agar inhibits the growth of gram-positive bacteria; it is used to distinguish among gram-negative bacteria. The culture from Monica's stool sample reveals that she has been infected with *Salmonella* bacteria. There are only two species of *Salmonella*: *S. enterica* and *S. bongori*. Monica's infection is caused by *S. enterica*; however, there are over 2500 serovars of *S. enterica* that can infect people. Upon receiving the results from the lab, Monica's nurse practitioner calls the Nevada Department of Health to inform them of her patient's diagnosis and to let them know that Monica's friend has the same symptoms. It is important that the department of health identify the serovar to determine whether there is an outbreak from one source and to trace that source.

How will the department of health identify the correct *S. enterica* serovar?

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Phage Typing

Like serological testing, phage typing looks for similarities among bacteria. Both techniques are useful in tracing the origin and course of a disease outbreak. **Phage typing** is a test for determining which phages a bacterium is susceptible to. Recall from Chapter 8 (page 234) that bacteriophages (phages) are bacterial viruses and that they usually cause lysis of the bacterial cells they infect. They are highly specialized, in that they usually infect only members of a particular species, or even particular strains within a species. One bacterial strain might be susceptible to two different phages, whereas another strain of the same species might be susceptible to those two phages plus a third phage. Bacteriophages will be discussed further in Chapter 13.

The sources of food-associated infections can be traced by phage typing. One version of this procedure starts with a plate totally covered with bacteria growing on agar. A drop of each different phage type to be used in the test is then placed on the bacteria. Wherever the phages are able to infect and lyse the bacterial cells, clearings in the bacterial growth (called plaques) appear (Figure 10.13). Such a test might show, for instance, that bacteria isolated from a surgical wound have the same pattern of phage sensitivity as those isolated from the operating surgeon or surgical nurses. This result establishes that the surgeon or a nurse is the source of infection.

Fatty Acid Profiles

Bacteria synthesize a wide variety of fatty acids, and in general, these fatty acids are constant for a particular species. Commercial systems have been designed to separate cellular fatty acids to compare them to fatty acid profiles of known organisms. Fatty acid profiles, called **FAME** (*f*atty *a*cid *m*ethyl *e*ster), are widely used in clinical and public health laboratories.

Flow Cytometry

Flow cytometry can be used to identify bacteria in a sample without culturing the bacteria. In a *flow cytometer,* a moving

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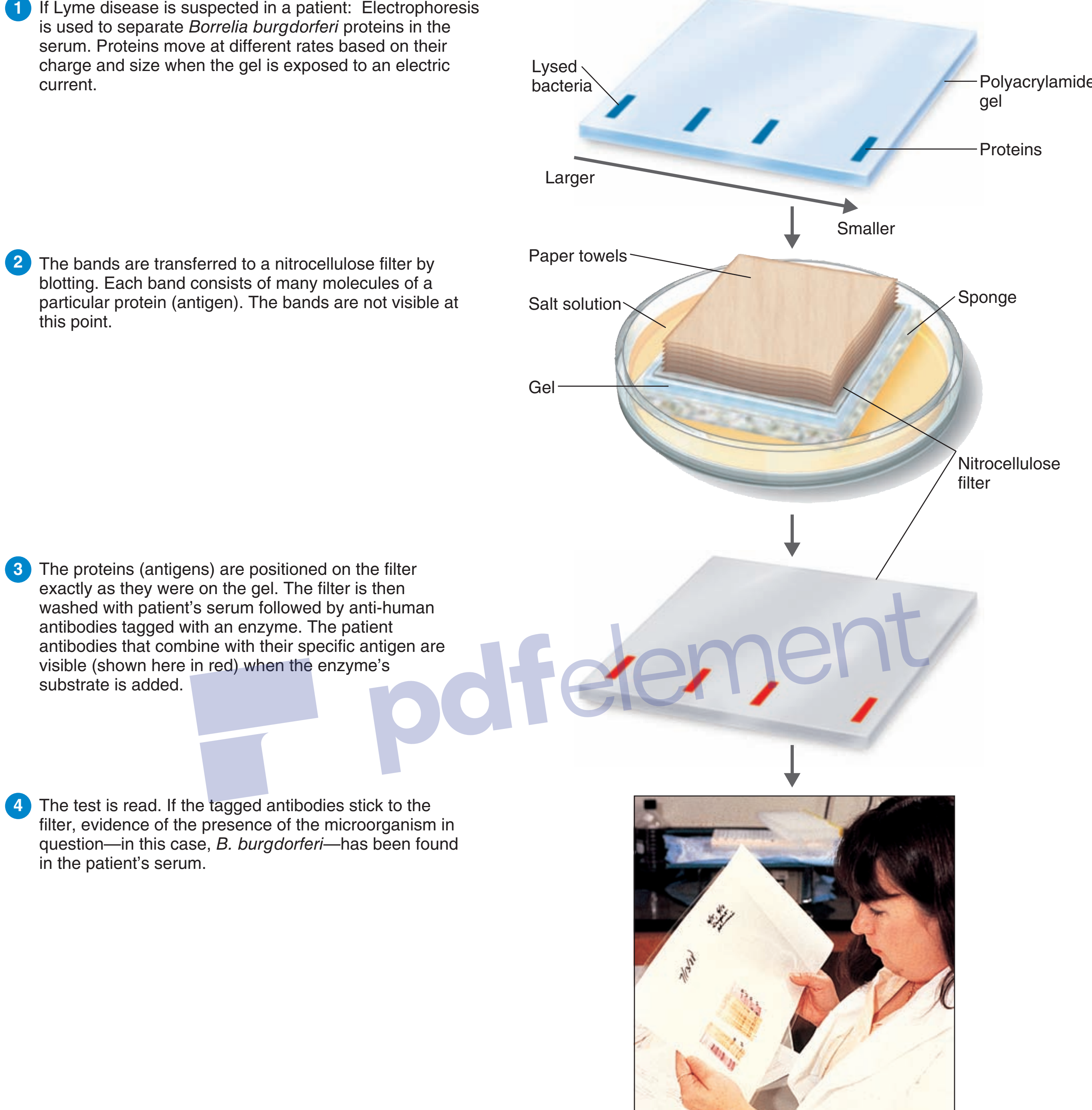

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Figure 10.12 The Western blot. Proteins separated by electrophoresis can be detected by their reactions with antibodies.

Q Name two diseases that may be diagnosed by Western blotting.

fluid containing bacteria is forced through a small opening (see Figure 18.12, page 521). The simplest method detects the presence of bacteria by detecting the difference in electrical conductivity between cells and the surrounding medium. If the fluid passing through the opening is illuminated by a laser, the scattering of light provides information about the cell size, shape, density, and surface, which is analyzed by a computer. Fluorescence can be used to detect naturally fluorescent cells, such as *Pseudomonas,* or cells tagged with fluorescent dyes.

Milk can be a vehicle for disease transmission. A proposed test that uses flow cytometry to detect *Listeria* in milk could save time because the bacteria would not need to be cultured for

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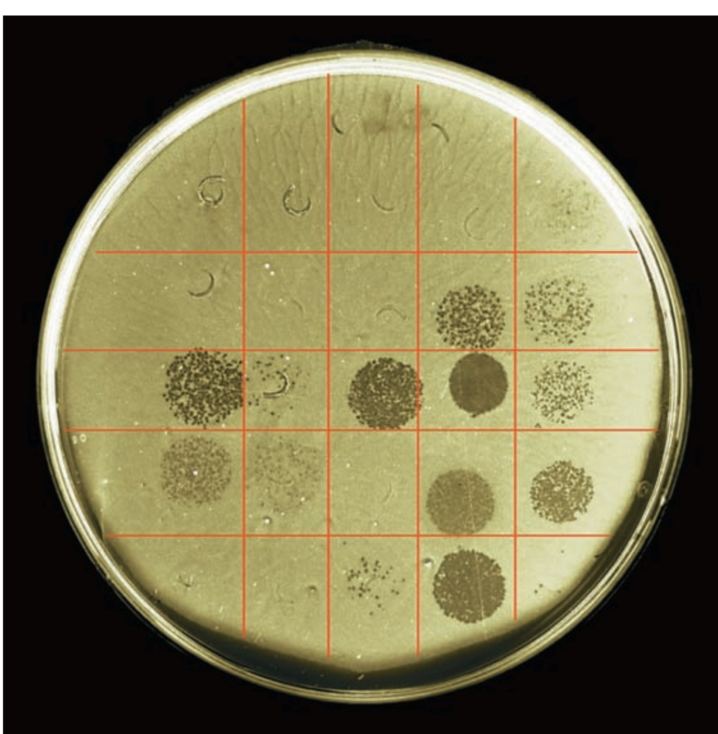

Figure 10.13 Phage typing of a strain of *Salmonella enterica*. The tested strain was grown over the entire plate. Plaques, or areas of lysis, were produced by bacteriophages, indicating that the strain was sensitive to infection by these phages. Phage typing is used to distinguish *S. enterica* serotypes and *Staphylococcus aureus* types.

Q What is being identified in phage typing?

identification. Antibodies against *Listeria* can be labeled with a fluorescent dye and added to the milk to be tested. The milk is passed through the flow cytometer, which records the fluorescence of the antibody-labeled cells.

DNA Base Composition

Taxonomists can use an organism's **DNA base composition** to draw conclusions about relatedness. This base composition is usually expressed as the percentage of guanine plus cytosine (G + C). The base composition of a single species is theoretically a fixed property; thus, a comparison of the G + C content in different species can reveal the degree of species relatedness. As we saw in Chapter 8, each guanine (G) in DNA has a complementary cytosine (C). Similarly, each adenine (A) in the DNA has a complementary thymine (T). Therefore, the percentage of DNA bases that are GC pairs also tells us the percentage that are AT pairs (GC + AT = 100%). Two organisms that are closely related and hence have many identical or similar genes will have similar amounts of the various bases in their DNA. However, if there is a difference of more than 10% in their percentage of GC pairs (for example, if one bacterium's DNA contains 40% GC and another bacterium has 60% GC), then these two organisms are probably not related. Of course, two organisms that have the same percentage of GC are not necessarily

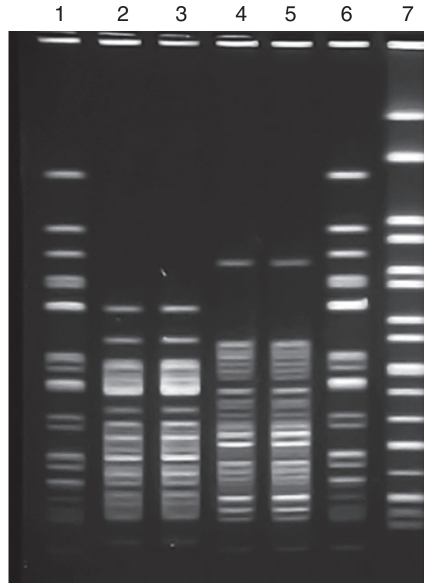

Figure 10.14 DNA fingerprints. DNA from seven different bacteria was digested with the same restriction enzyme. Each digest was put in a different well (origin) in the agarose gel. An electrical current was then applied to the gel to separate the fragments by size and electrical charge. The DNA was made visible by staining with a dye that fluoresces under ultraviolet light. Comparison of the lanes shows that DNA samples (and therefore the bacteria) in lanes 2 and 3; 4 and 5; and 1 and 6 are identical.

Q What is an RFLP?

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closely related; other supporting data are needed to draw conclusions about their phylogenetic relationship.

DNA Fingerprinting

Determining the entire sequence of bases in an organism's DNA is currently impractical for laboratory identification because of the great amount of time required. However, the use of restriction enzymes enables researchers to compare the base sequences of different organisms. Restriction enzymes cut a molecule of DNA everywhere a specific base sequence occurs, producing restriction fragments (as discussed in Chapter 9, page 247). For example, the enzyme *Eco*RI cuts DNA at the arrows in the sequence

$$\ldots G^{\downarrow}A\,A\,T\,T\,C \ldots$$
$$\ldots C\,T\,T\,A\,A_{\uparrow}G \ldots$$

In this technique, the DNA from two microorganisms is treated with the same restriction enzyme, and the restriction fragments (RFLPs) produced are separated by electrophoresis producing a **DNA fingerprint** (see Figure 9.17, page 263). A comparison of the number and sizes of restriction fragments that are produced from different organisms provides information about their genetic similarities and differences; the more similar the patterns, or *DNA fingerprints*, the more closely related the organisms are expected to be (**Figure 10.14**).

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DNA fingerprinting is used to determine the source of hospital-acquired infections. In one hospital, patients undergoing coronary-bypass surgery developed infections caused by *Rhodococcus bronchialis* (rō-dō-kok′kus bron-kē′al-is). The DNA fingerprints of the patients' bacteria and the bacteria of one nurse were identical. The hospital was thus able to break the chain of transmission of this infection by encouraging this nurse to use aseptic technique.

This has led to interest in finding a few genes that are present in all species and provide a large variation between species. Primers for these genes would be used for PCR to produce a *DNA bar code* for each species. This was first proposed in 2003 for eukaryotic species, but the necessary six to nine genes for bacterial identification have not been found.

Clinical Case

Salmonella serovars are identified by serotyping with antisera against previously isolated serovars. The department of health identifies the serovar; Monica and her friend are infected with *Salmonella tennessee* bacteria. By now, the department of health has been inundated with calls; 27 additional cases of *Salmonella tennessee* infection have been identified and reported from all over the state of Nevada.

How can the department of health determine whether these 29 cases are related?

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Nucleic Acid Amplification Tests (NAATs)

When a microorganism cannot be cultured by conventional methods, the causative agent of an infectious disease might not be recognized. However, **nucleic acid amplification tests (NAATs)** can be used to increase the amount of microbial DNA to levels that can be tested by gel electrophoresis. NAATs use PCR, reverse-transcription PCR, and real-time PCR (see Chapter 9, page 251). If a primer for a specific microorganism is used, the presence of amplified DNA indicates that microorganism is present.

In 1992, researchers used PCR to determine the causative agent of Whipple's disease, which was previously an unknown bacterium now named *Tropheryma whipplei* (trō′fėr-ē-mä whip′plē-e). Whipple's disease was first described in 1907 by George Whipple as a gastrointestinal and nervous system disorder caused by an unknown bacillus. No one has been able to culture the bacterium to identify it, and thus PCR provides the only reliable methods of diagnosing and treating the disease.

In recent years, PCR made possible several discoveries. For example, in 1992, Raul Cano used PCR to amplify DNA from *Bacillus* bacteria in amber that was 25 to 40 million years old. These primers were made from rRNA sequences in living *B. circulans* to amplify DNA coding for rRNA in the amber. These primers will cause amplification of DNA from other *Bacillus* species but do not cause amplification of DNA from other bacteria that might have been present, such as *Escherichia* or *Pseudomonas*. The DNA was sequenced after amplification. This information was used to determine the relationships between the ancient bacteria and modern bacteria.

In 1993, microbiologists identified a *Hantavirus* as the cause of an outbreak of hemorrhagic fever in the American Southwest using PCR. The identification was made in record time—less than 2 weeks. PCR was used in 1994 to identify the causative agent of a new tickborne disease (human granulocytic ehrlichiosis) as the bacterium *Ehrlichia chaffeensis* (ėr′lik-ē-ä chaf′fē-en-sis) (page 660). PCR is used to identify the source of rabies viruses; see the box in Chapter 22 (page 631).

In 2009, public health scientists used real-time PCR to identify a new strain of H1N1 influenza virus.

Nucleic Acid Hybridization

If a double-stranded molecule of DNA is subjected to heat, the complementary strands will separate as the hydrogen bonds between the bases break. If the single strands are then cooled slowly, they will reunite to form a double-stranded molecule identical to the original double strand. (This reunion occurs because the single strands have complementary sequences.) When this technique is applied to separated DNA strands from two different organisms, it is possible to determine the extent of similarity between the base sequences of the two organisms. This method is known as **nucleic acid hybridization.** The procedure assumes that if two species are similar or related, a major portion of their nucleic acid sequences will also be similar. The procedure measures the ability of DNA strands from one organism to hybridize (bind through complementary base pairing) with the DNA strands of another organism (**Figure 10.15**). The greater the degree of hybridization, the greater the degree of relatedness.

Similar hybridization reactions can occur between any single-stranded nucleic acid chain: DNA-DNA, RNA-RNA, DNA-RNA. An RNA transcript will hybridize with the separated template DNA to form a DNA-RNA hybrid molecule. Nucleic acid hybridization reactions are the basis of several techniques (described below) that are used to detect the presence of microorganisms and to identify unknown organisms.

Southern Blotting

Nucleic acid hybridization can be used to identify unknown microorganisms by **Southern blotting** (see Figure 9.16, page 262). In addition, rapid identification methods using **DNA probes** are being developed. One method involves breaking DNA extracted from *Salmonella* into fragments with a restriction enzyme, then selecting a specific fragment as the probe for *Salmonella* (**Figure 10.16**). This fragment must be able to hybridize with the

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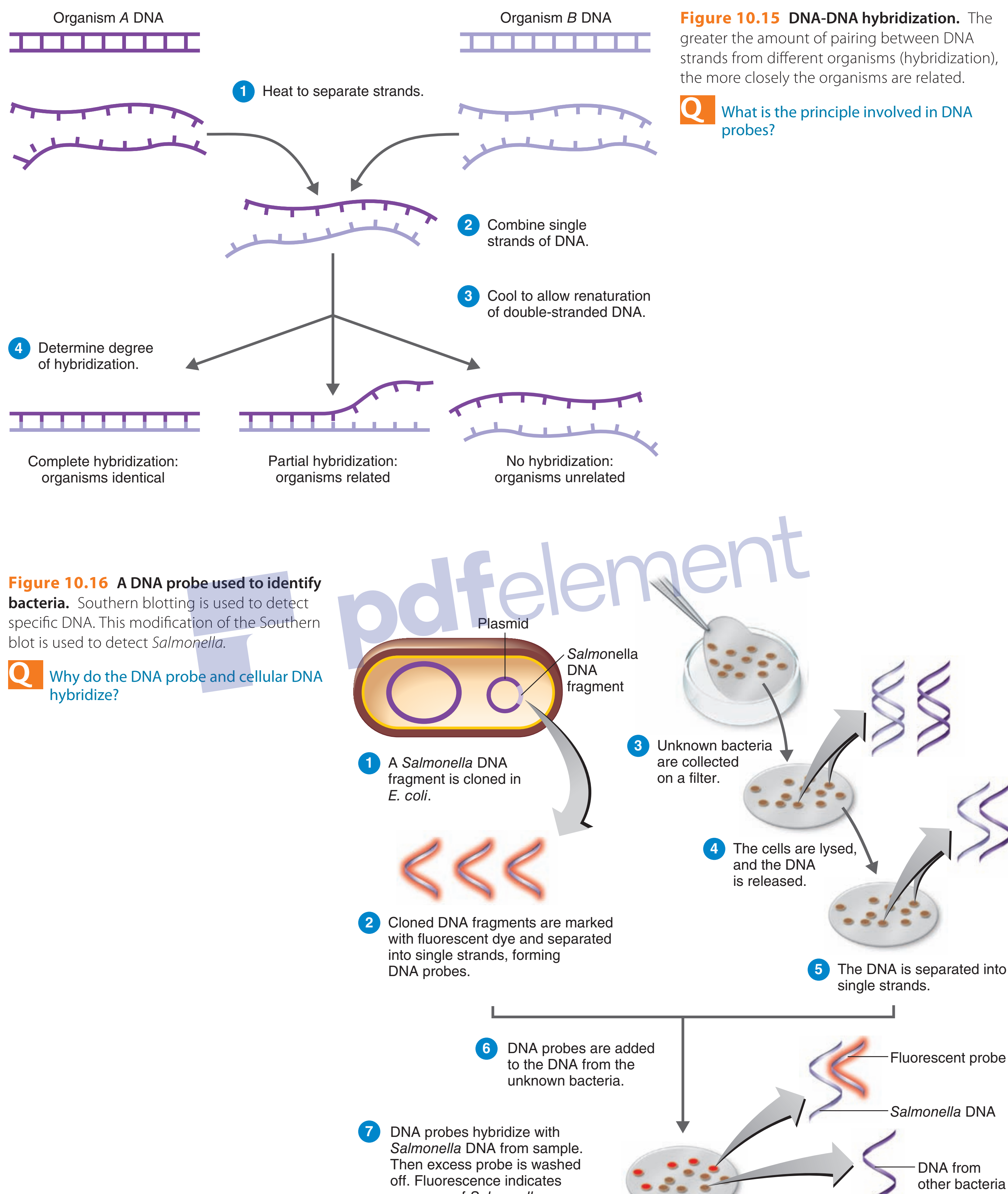

Figure 10.15 DNA-DNA hybridization. The greater the amount of pairing between DNA strands from different organisms (hybridization), the more closely the organisms are related.

Q What is the principle involved in DNA probes?

Figure 10.16 A DNA probe used to identify bacteria. Southern blotting is used to detect specific DNA. This modification of the Southern blot is used to detect *Salmonella.*

Q Why do the DNA probe and cellular DNA hybridize?

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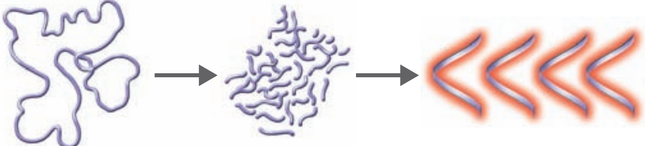
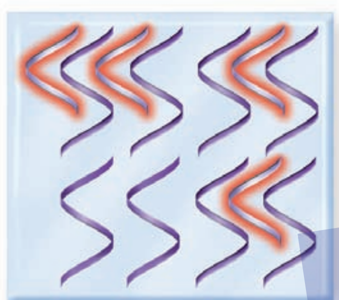
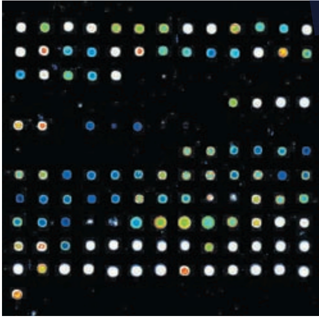
(a) A DNA chip can be manufactured to contain hundreds of thousands of synthetic single-stranded DNA sequences. Assume that each DNA sequence was unique to a different gene.



(b) Unknown DNA from a sample is separated into single strands, enzymatically cut, and labeled with a fluorescent dye.



(c) The unknown DNA is inserted into the chip and allowed to hybridize with the DNA on the chip.



(d) The tagged DNA will bind only to the complementary DNA on the chip. The bound DNA will be detected by its fluorescent dye and analyzed by a computer. In this *Salmonella* antimicrobial resistance gene microarray, *S. typhimurium*-specific antibiotic resistance gene probes are green, *S. typhi*-specific resistance gene probes are red, and antibiotic-resistance genes found in both serovars appear yellow/orange.

Figure 10.17 DNA chip. This DNA chip contains probes for antibiotic-resistance genes. It is used to detect antibiotic-resistant bacteria in samples collected from animals on a farm or in slaughter facilities.

What is on the chip to make it specific for a particular micro-organism?

DNA of all *Salmonella* strains, but not with the DNA of closely related enteric bacteria.

DNA Chips

An exciting new technology is the **DNA chip,** or **microarray,** which can quickly detect a pathogen in a host or the environment by identifying a gene that is unique to that pathogen (Figure 10.17). The DNA chip is composed of DNA probes. A sample containing DNA from an unknown organism is labeled with a fluorescent dye and added to the chip. Hybridization between the probe DNA and DNA in the sample is detected by fluorescence.

Ribotyping and Ribosomal RNA Sequencing

Ribotyping is currently being used to determine the phylogenetic relationships among organisms. There are several advantages to using rRNA. First, all cells contain ribosomes. Second, RNA genes have undergone few changes over time so all members of a domain, phylum, and, in some cases, a genus, have the same "signature" sequences in their rRNA. The rRNA used most often is a component of the smaller portion of ribosomes. A third advantage of rRNA sequencing is that cells do not have to be cultured in the laboratory.

DNA can be amplified by PCR using an rRNA primer for specific signature sequences. The amplified fragments are subsequently cut with one or more restriction enzymes and separated by electrophoresis. The resulting band patterns can then be compared. Then the rRNA genes in the amplified fragments can be sequenced to determine evolutionary relationships between organisms. This technique is useful for classifying a newly discovered organism to domain or phylum or to determine the general types of organisms present in one environment. More specific probes (see page 255) are needed to identify individual species, however.

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Fluorescent In Situ Hybridization (FISH)

Fluorescent dye-labeled RNA or DNA probes are used to specifically stain microorganisms in place, or in situ. This technique is called **fluorescent in situ hybridization,** or **FISH.** Cells are treated so the probe enters the cells and reacts with target DNA in the cell (in situ). FISH is used to determine the identity, abundance, and relative activity of microorganisms in an environment and can be used to detect bacteria that have not yet been cultured. Using FISH, a tiny bacterium, *Pelagibacter* (pel-aj′ ē-bak-tėr), was discovered in the ocean and determined to be related to the rickettsias (page 304). As probes are developed, FISH can be used to detect bacteria in drinking water or bacteria in a patient without the normal 24-hour or longer wait required for culturing the bacteria (Figure 10.18).

Putting Classification Methods Together

Morphological characteristics, differential staining, and biochemical testing were the only identification tools available just a few years ago. Technological advancements are making it possible to use nucleic acid analysis techniques, once reserved for classification, for routine identification. Information obtained about microbes is used to identify and classify the organisms. Two methods of using the information are described on the facing page.

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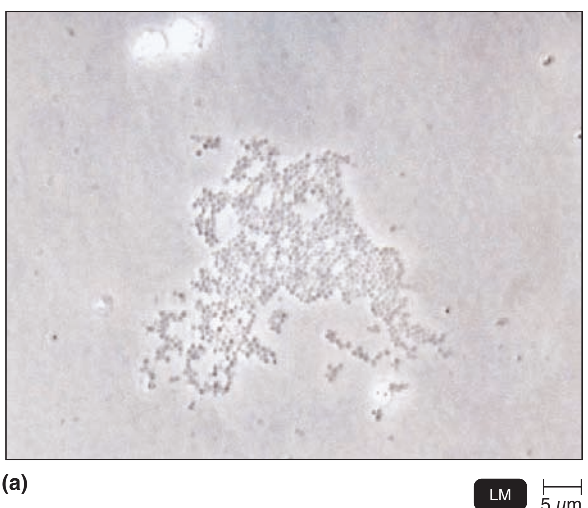


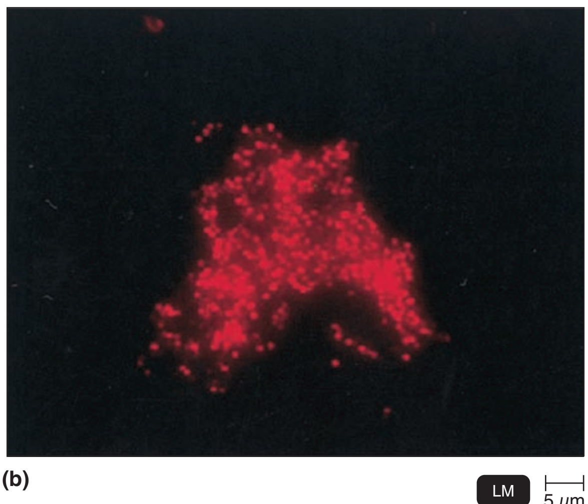
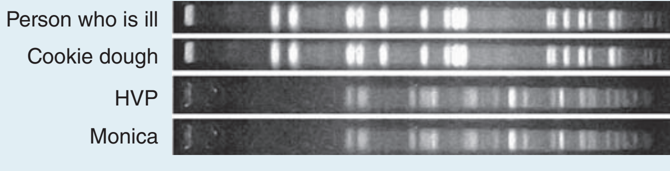


Figure 10.18 FISH, or fluorescent in situ hybridization. A DNA or RNA probe attached to fluorescent dyes is used to identify chromosomes. Bacteria seen with phase-contrast microscopy (**a**) are identified with a fluorescent-labeled probe that hybridizes with a specific sequence of DNA in *Staphylococcus aureus* (**b**).

Q What is stained using the FISH technique?

Clinical Case

Salmonella isolates from each of the 29 infected people are sent to the state's public health laboratory for DNA fingerprinting. The DNA fingerprints are then sent to the Centers for Disease Control and Prevention (CDC). At the CDC, computer software compares each of the *Salmonella* DNA fingerprints to determine whether all 29 cases of *Salmonella tennessee* are identical. At this point, the CDC has received over 400 samples from 20 states, indicating a potential nationwide outbreak. Below is a figure of Monica's *Salmonella* DNA fingerprint along with other DNA fingerprint samples.



What can the CDC conclude about the outbreak based on these DNA fingerprints?

273 286 287 290 **293** 294

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Dichotomous Keys

Dichotomous keys are widely used for identification. In a dichotomous key, identification is based on successive questions, and each question has two possible answers (*dichotomous* means cut in two). After answering one question, the investigator is directed to another question until an organism is identified. Although these keys often have little to do with phylogenetic relationships, they are invaluable for identification. For example, a dichotomous key for bacteria could begin with an easily determined characteristic, such as cell shape, and move on to the ability to ferment a sugar. Dichotomous keys are shown in Figure 10.8 and in the box on page 282. MM **Animations** Dichotomous Keys: Overview, Sample with Flowchart, Practice

Cladograms

Cladograms are maps that show evolutionary relationships among organisms (*clado-* means branch). Cladograms are shown in Figures 10.1 and 10.6. Each branch point on the cladogram is defined by a feature shared by various species on that branch. Historically, cladograms for vertebrates were made using fossil evidence; however, rRNA sequences are now being used to confirm assumptions based on fossils. As we said earlier, most microorganisms do not leave fossils; therefore, rRNA sequencing is primarily used to make cladograms for microorganisms. The small rRNA subunit used has 1500 bases, and computer programs do the calculations. The steps for constructing a cladogram are shown in **Figure 10.19**.

1. Two rRNA sequences are aligned, and
2. the percentage of similarity between the sequences is calculated.
3. Then the horizontal branches are drawn in a length proportional to the calculated percent similarity. All species beyond a node (branch point) have similar rRNA sequences, suggesting that they arose from an ancestor at that node.

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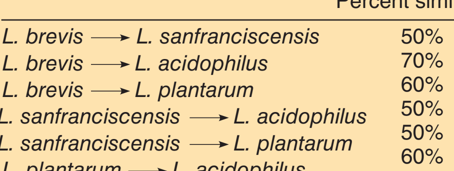
1 Determine the sequence of bases in an rRNA molecule for each organism. Only a short sequence of bases is shown for this example.

Lactobacillus brevis	AGUCCAGAGC
L. sanfranciscensis	GUAAAAGAGC
L. acidophilus	AGCGGAGAGC
L. plantarum	ACGUUAGAGC

2 Calculate the percentage of similarity in the nucleotide bases between pairs of species. For example, there is a 70% similarity between the sequences for *L. brevis* and *L. acidophilus*.



3 Construct a cladogram. The length of the horizontal lines corresponds to the percent similarity values. Each branch point, or node, in the cladogram represents an ancestor common to all species beyond that node. Each node is defined by a similarity in rRNA present in all species beyond that branch point.

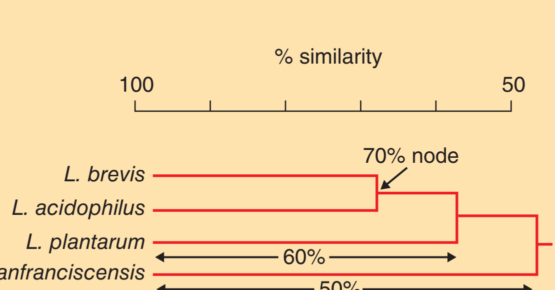


Figure 10.19 Building a cladogram.

Q Why do *L. brevis* and *L. acidophilus* branch from the same node?

Clinical Case Resolved

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Early in this outbreak, there was a cluster of *Salmonella tennessee* illness due to consumption of raw eggs. Ill people and randomly chosen uninfected people completed questionnaires about foods they ate. The ill people were significantly more likely than well persons to report eating raw cookie dough, which contains uncooked eggs. However, the CDC soon determines that the cookie dough cluster involves a different strain of *Salmonella tennessee* from the strain involved in the current outbreak. This strain is associated with hydrolyzed vegetable protein (HVP), a flavor enhancer that is commonly used in a variety of foods, including a vegetable dip and chips that Monica and her friend ate the day before they became ill. In conjunction with the CDC and the U.S. Food and Drug Administration, the manufacturer recalls that particular batch of HVP. Monica and her friend fully recover after a few days.

Tracing *Salmonella* infections to their source is essential because *Salmonella* can be transmitted through a variety of foods. It causes an estimated 1.4 million illnesses and 400 deaths annually in the United States.

DNA fingerprinting is currently used worldwide by public health laboratories to distinguish among *Salmonella* strains. NAATs are highly sensitive and specific, but primers or probes would have to be made for every strain. DNA fingerprinting can also detect strains because the RFLPs are made from the entire genome rather than from amplification of a few nucleotide sequences.

273 286 287 290 293 **294**

CHECK YOUR UNDERSTANDING

- What is in *Bergey's Manual*? **10-13**
- Design a rapid test for a *Staphylococcus aureus*. (*Hint:* See Figure 6.10, page 166.) **10-14**
- What is tested in Western blotting and Southern blotting? **10-15**
- What is identified by phage typing? **10-16**
- Why does PCR identify a microbe? **10-17**
- Which techniques involve nucleic acid hybridization? **10-18**
- Is a cladogram used for identification or classification? **10-12**, **10-19**

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Study Outline

Test your understanding with quizzes, microbe review, and a chapter post-test at www.masteringmicrobiology.com.

Introduction (p. 272)

1. Taxonomy is the science of the classification of organisms. Its goal is to show relationships among organisms.
2. Taxonomy also provides a means of identifying organisms.

The Study of Phylogenetic Relationships (pp. 273–277)

1. Phylogeny is the evolutionary history of a group of organisms.
2. The taxonomic hierarchy shows evolutionary, or phylogenetic, relationships among organisms.
3. Bacteria were separated into the Kingdom Prokaryotae in 1968.
4. Living organisms were divided into five kingdoms in 1969.

The Three Domains (pp. 273–275)

5. Living organisms are currently classified into three domains. A domain can be divided into kingdoms.
6. In this system, plants, animals, and fungi belong to the Domain Eukarya.
7. Bacteria (with peptidoglycan) form a second domain.
8. Archaea (with unusual cell walls) are placed in the Domain Archaea.

A Phylogenetic Hierarchy (pp. 275–277)

9. Organisms are grouped into taxa according to phylogenetic relationships (from a common ancestor).
10. Some of the information for eukaryotic relationships is obtained from the fossil record.
11. Prokaryotic relationships are determined by rRNA sequencing.

Classification of Organisms (pp. 277–281)

Scientific Nomenclature (p. 278)

1. According to scientific nomenclature, each organism is assigned two names, or a binomial: a genus and a specific epithet, or species.
2. Rules for assigning names to bacteria are established by the International Committee on Systematics of Prokaryotes.
3. Rules for naming fungi and algae are published in the *International Code of Botanical Nomenclature.*
4. Rules for naming protozoa are found in the *International Code of Zoological Nomenclature.*

The Taxonomic Hierarchy (p. 278)

5. A eukaryotic species is a group of organisms that interbreed with each other but do not breed with individuals of another species.
6. Similar species are grouped into a genus; similar genera are grouped into a family; families, into an order; orders, into a class; classes, into a phylum; phyla, into a kingdom; and kingdoms, into a domain.

Classification of Prokaryotes (pp. 278–280)

7. *Bergey's Manual of Systematic Bacteriology* is the standard reference on bacterial classification.
8. A group of bacteria derived from a single cell is called a strain.
9. Closely related strains constitute a bacterial species.

Classification of Eukaryotes (pp. 280–281)

10. Eukaryotic organisms may be classified into the Kingdom Fungi, Plantae, or Animalia.
11. Protists are mostly unicellular organisms; these organisms are currently being assigned to kingdoms.
12. Fungi are absorptive chemoheterotrophs that develop from spores.
13. Multicellular photoautotrophs are placed in the Kingdom Plantae.
14. Multicellular ingestive heterotrophs are classified as Animalia.

Classification of Viruses (p. 281)

15. Viruses are not placed in a kingdom. They are not composed of cells and cannot grow without a host cell.
16. A viral species is a population of viruses with similar characteristics that occupies a particular ecological niche.

Methods of Classifying and Identifying Microorganisms (pp. 281–294)

1. *Bergey's Manual of Determinative Bacteriology* is the standard reference for laboratory identification of bacteria.
2. Morphological characteristics are useful in identifying microorganisms, especially when aided by differential staining techniques.
3. The presence of various enzymes, as determined by biochemical tests, is used in identifying bacteria and yeasts.
4. Serological tests, involving the reactions of microorganisms with specific antibodies, are useful in determining the identity of strains and species, as well as relationships among organisms. ELISA and Western blotting are examples of serological tests.
5. Phage typing is the identification of bacterial species and strains by determining their susceptibility to various phages.
6. Fatty acid profiles can be used to identify some organisms.
7. Flow cytometry measures physical and chemical characteristics of cells.
8. The percentage of GC base pairs in the nucleic acid of cells can be used in the classification of organisms.
9. The number and sizes of DNA fragments, or DNA fingerprints, produced by restriction enzymes are used to determine genetic similarities.
10. NAATs can be used to amplify a small amount of microbial DNA in a sample. The presence or identification of an organism is indicated by amplified DNA.
11. Single strands of DNA, or of DNA and RNA, from related organisms will hydrogen-bond to form a double-stranded molecule; this bonding is called nucleic acid hybridization.
12. Southern blotting, DNA chips, and FISH are examples of nucleic acid hybridization techniques.
13. The sequence of bases in ribosomal RNA can be used in the classification of organisms.
14. Dichotomous keys are used for the identification of organisms. Cladograms show phylogenetic relationships among organisms.

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Study Questions

Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

1. Which of the following organisms are most closely related? Are any two the same species? On what did you base your answer?

Characteristic	A	B	C	D
Morphology	Rod	Coccus	Rod	Rod
Gram Reaction	+	–	–	+
Glucose Utilization	Fermentative	Oxidative	Fermentative	Fermentative
Cytochrome Oxidase	Present	Present	Absent	Absent
GC Moles %	48–52	23–40	50–54	49–53

2. Here is some additional information on the organisms in question 1:

Organism	% DNA Hybridization
A and B	5–15
A and C	5–15
A and D	70–90
B and C	10–20
B and D	2–5

Which of these organisms are most closely related? Compare this answer with your response to review question 1.

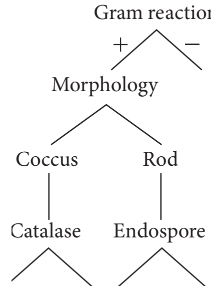
3. DRAW IT Use the additional information below to construct a cladogram for some of the organisms used in question 4. What is the purpose of a cladogram? How does your cladogram differ from a dichotomous key for these organisms?

	Similarity in rRNA Bases
P. aeruginosa—M. pneumoniae	52%
P. aeruginosa—C. botulinum	52%
P. aeruginosa—E. coli	79%
M. pneumoniae—C. botulinum	65%
M. pneumoniae—E. coli	52%
E. coli—C. botulinum	52%

% similarity

100 50

4. DRAW IT Use the information in the table below to complete the dichotomous key to these organisms. What is the purpose of a dichotomous key? Look up each genus in Chapter 11, and provide an example of why this organism is of interest to humans.



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	Morphology	Gram Reaction	Acid from Glucose	Growth in Air (21% O_2)	Motile by Peritrichous Flagella	Presence of Cytochrome Oxidase	Produce Catalase
Staphylococcus aureus	Coccus	+	+	+	–	–	+
Streptococcus pyogenes	Coccus	+	+	+	–	–	–
Mycoplasma pneumoniae	Coccus	–	+	+ (Colonies < 1 mm)	–	–	+
Clostridium botulinum	Rod	+	+	–	+	–	–
Escherichia coli	Rod	–	+	+	+	–	+
Pseudomonas aeruginosa	Rod	–	+	+	–	+	+
Campylobacter fetus	Vibrio	–	–	–	–	+	+
Listeria monocytogenes	Rod	+	+	+	+	–	+

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5. NAME IT Use the key in the Applications of Microbiology box on page 282 to identify the gram-negative rod causing pneumonia in a sea otter. It is V–P negative, indole-negative, and urease-positive.

Multiple Choice

1. *Bergey's Manual of Systematic Bacteriology* differs from *Bergey's Manual of Determinative Bacteriology* in that the former
   - **a.** groups bacteria into species.
   - **b.** groups bacteria according to phylogenetic relationships.
   - **c.** groups bacteria according to pathogenic properties.
   - **d.** groups bacteria into 19 species.
   - **e.** all of the above
2. *Bacillus* and *Lactobacillus* are not in the same order. This indicates that which one of the following is *not* sufficient to assign an organism to a taxon?
   - **a.** biochemical characteristics
   - **b.** amino acid sequencing
   - **c.** phage typing
   - **d.** serology
   - **e.** morphological characteristics
3. Which of the following is used to classify organisms into the Kingdom Fungi?
   - **a.** ability to photosynthesize; possess a cell wall
   - **b.** unicellular; possess cell wall; prokaryotic
   - **c.** unicellular; lacking cell wall; eukaryotic
   - **d.** absorptive; possess cell wall; eukaryotic
   - **e.** ingestive; lacking cell wall; multicellular; prokaryotic
4. Which of the following is *false* about scientific nomenclature?
   - **a.** Each name is specific.
   - **b.** Names vary with geographical location.
   - **c.** The names are standardized.
   - **d.** Each name consists of a genus and specific epithet.
   - **e.** It was first designed by Linnaeus.
5. You could identify an unknown bacterium by all of the following *except*
   - **a.** hybridizing a DNA probe from a known bacterium with the unknown's DNA.
   - **b.** making a fatty acid profile of the unknown.
   - **c.** specific antiserum agglutinating the unknown.
   - **d.** ribosomal RNA sequencing.
   - **e.** percentage of guanine + cytosine.
6. The wall-less mycoplasmas are considered to be related to gram-positive bacteria. Which of the following would provide the most compelling evidence for this?
   - **a.** They share common rRNA sequences.
   - **b.** Some gram-positive bacteria and some mycoplasmas produce catalase.
   - **c.** Both groups are prokaryotic.
   - **d.** Some gram-positive bacteria and some mycoplasmas have coccus-shaped cells.
   - **e.** Both groups contain human pathogens.

Use the following choices to answer questions 7 and 8.

- **a.** Animalia
- **b.** Fungi
- **c.** Plantae
- **d.** Firmicutes (gram-positive bacteria)
- **e.** Proteobacteria (gram-negative bacteria)

7. Into which group would you place a multicellular organism that has a mouth and lives inside the human liver?
8. Into which group would you place a photosynthetic organism that lacks a nucleus and has a thin peptidoglycan wall surrounded by an outer membrane?

Use the following choices to answer questions 9 and 10.

- **1.** 9 + 2 flagella
- **2.** 70S ribosome
- **3.** fimbria
- **4.** nucleus
- **5.** peptidoglycan
- **6.** plasma membrane

9. Which is (are) found in all three domains?
   - **a.** 2, 6
   - **b.** 5
   - **c.** 2, 4, 6
   - **d.** 1, 3, 5
   - **e.** all six
10. Which is (are) found *only* in prokaryotes?
   - **a.** 1, 4, 6
   - **b.** 3, 5
   - **c.** 1, 2
   - **d.** 4
   - **e.** 2, 4, 5

Critical Thinking

1. The GC content of *Micrococcus* is 66–75 moles %, and of *Staphylococcus*, 30–40 moles %. According to this information, would you conclude that these two genera are closely related?
2. Describe the use of a DNA probe and PCR for:
   - **a.** rapid identification of an unknown bacterium.
   - **b.** determining which of a group of bacteria are most closely related.
3. SF medium is a selective medium, developed in the 1940s, to test for fecal contamination of milk and water. Only certain gram-positive cocci can grow in this medium. Why is it named SF? Using this medium, which genus will you culture? (*Hint:* Refer to page 278.)

Clinical Applications

1. A 55-year-old veterinarian was admitted to a hospital with a 2-day history of fever, chest pain, and cough. Gram-positive cocci were detected in his sputum, and he was treated for lobar pneumonia with penicillin. The next day, another Gram stain of his sputum revealed gram-negative rods, and he was switched to ampicillin and gentamicin. A sputum culture showed biochemically inactive gram-negative rods identified as *Pantoea (Enterobacter) agglomerans.* After fluorescent-antibody staining and phage typing, *Yersinia pestis* was identified in the patient's sputum and blood, and chloramphenicol and tetracycline were administered. The patient died 3 days after admission to the hospital. Tetracycline was given to his 220 contacts (hospital personnel, family, and co-workers). What disease did the patient have? Discuss what went wrong in the diagnosis and how his death might have been prevented. Why were the 220 other people treated? (*Hint:* Refer to Chapter 23.)

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2. A 6-year-old girl was admitted to a hospital with endocarditis. Blood cultures showed a gram-positive, aerobic rod identified by the hospital laboratory as *Corynebacterium xerosis.* The girl died after 6 weeks of treatment with intravenous penicillin and chloramphenicol. The bacterium was tested by another laboratory and identified as *C. diphtheriae.* The following test results were obtained by each laboratory:

	Hospital Lab	Other Lab
Catalase	+	+
Nitrate reduction	+	+
Urea	–	–
Esculin hydrolysis	–	–
Glucose fermentation	+	+
Sucrose fermentation	–	+
Serological test for toxin production	Not done	+

Provide a possible explanation for the incorrect identification. What are the potential public health consequences of misidentifying *C. diphtheriae*? (*Hint:* Refer to Chapter 24.)

3. Using the following information, create a dichotomous key for distinguishing these unicellular organisms. Which cause human disease?

	Mitochondria?	Chlorophyll?	Nutritional Type?	Motile?
Euglena	+	+	Both	+
Giardia	–	–	Heterotroph	+
Nosema	–	–	Heterotroph	–
Pfiesteria	+	+	Autotroph	+
Trichomonas	–	–	Heterotroph	+
Trypanosoma	+	–	Heterotroph	+

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Using the additional information shown below, create a dichotomous key for these organisms. Do your two keys differ? Explain why. Which key is more useful for laboratory identification? For classification?

	rRNA base #																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Euglena	C	C	A	G	G	U	U	G	U	U	C	C	A	G	U	U	U	U	A	A
Giardia	C	C	A	U	A	U	U	U	U	U	G	A	C	G	A	A	G	G	U	C
Nosema	C	C	A	U	A	U	U	U	U	U	A	A	C	G	A	A	G	G	C	C
Pfiesteria	C	C	A	A	C	U	U	A	U	U	C	C	A	G	U	U	U	C	A	G
Trichomonas	C	C	A	U	A	U	U	U	U	U	G	A	C	G	A	A	G	G	G	C
Trypanosoma	C	C	A	C	G	U	U	G	U	U	C	C	A	G	U	U	U	A	A	A