CHAPTER 9

AN INTRODUCTION TO TAXONOMY: THE BACTERIA

Exploring deep-sea geography, you might notice giant tubeworms (up to 2 meters tall) and other interesting invertebrates living in habitats near geothermal vents. But how do these organisms survive in locations that appear so hostile?

The answer is that invertebrates like the giant tubeworm have formed symbiotic relationships with members of Archaea, so that products from the metabolic processes of the microorganisms nourish the macroorganism. For example, the chemolithotrophic bacteria have been found in the modified gastrointestinal tract of giant tubeworms (giant tubeworms lack a mouth, gut, or anus). These chemolithotrophs have metabolisms that fix inorganic sources (CO_3^-, HCO_3^-) into organic carbon sources via the same enzymes utilized in the Calvin cycle of certain autotrophs. The tubeworms are then able to use the organic carbon sources in cellular processes.

What do the tubeworms do for the bacteria? The tubeworms have well-vascularized plumes that trap O_2 and H_2S from the thermal vents and transport those substances to the chemolithotrophs. The bacteria use the O_2 and H_2S in their life-sustaining energy reactions. It's an exquisite partnership!

Humans appear to have an innate need to name things. In many primitive societies, a person who knows the true name of an object or of another person is believed to have power over that object or person. Naming helps us to understand our world and to communicate with others about it.

TAXONOMY: THE SCIENCE OF CLASSIFICATION

In science, accurate and standardized names are essential. All chemists must mean the same thing when they talk about an element or a compound; physicists must agree on terms when they discuss matter or energy; and biologists must agree on the names of organisms, be they tigers or bacteria.

Faced with the great number and diversity of organisms, biologists use the characteristics of different organisms to describe specific forms of life and to identify new ones. The grouping of related organisms together is the basis of *classification*. The most obvious reasons for classification are (1) to establish the criteria for identifying organisms, (2) to arrange related organisms into groups, and (3) to provide important information on how organisms evolved. **Taxonomy** is the science of classification. It provides an orderly basis for the naming of organisms and for placing organisms into a category, or **taxon** (plural: *taxa*).

Another important aspect of taxonomy is that it makes use of and makes sense of the fundamental concepts of unity and diversity among living things. Organisms classified in any particular group have certain common characteristics—that is, they have unity with respect to these characteristics. For example, humans walk upright and have a well-developed brain; *Escherichia coli* cells are rod-shaped and have a Gram-negative cell wall. The organisms within taxonomic groups exhibit diversity as well. Even members of the same species display variations in size, shape, and other characteristics. Humans vary in height, weight, hair and eye color, and facial features. Certain kinds of bacteria vary somewhat in shape and in their ability to form specific structures, such as endospores.

A basic principle of taxonomy is that members of higher-level groups share fewer characteristics than those in lower-level groups. Like all other vertebrates, humans have backbones, but humans share fewer characteristics with fish and birds than with other mammals. Likewise, nearly all bacteria have a cell wall, but in some the wall is Gram-positive and in others it is Gram-negative.

Linnaeus, the Father of Taxonomy

The eighteenth-century Swedish botanist Carolus Linnaeus is credited with founding the science of taxonomy (Figure 9.1). He originated **binomial nomenclature**, the system that is still used today to name all living things. In the binomial, or "two-name," system, the first name designates the **genus** (plural: *genera*) of an organism, and its first letter is capitalized. The second name is the **specific epithet**, and it is not capitalized even when derived from the name of the person who discovered it. Together the genus and specific epithet identify the **species** to which the organism belongs. Both words are italicized in print but underlined when handwritten. When there is no danger of confusion, the genus name may be abbreviated to a single letter. Thus, *Escherichia coli* is often written *E. coli*, and humans (*Homo sapiens*) may be identified as *H. sapiens*.

Why taxonomy? Common names are confusing. Passer domesticus is the English sparrow in America, house sparrow in England, gorrion in Spain, musch in Holland, and hussparf in Sweden.

The name of an organism often tells something about it, such as its shape, where it is found, what nutrients it uses, who discovered it, or what disease it causes. Some examples of names and their meanings are shown in Table 9.1.

The members of a species generally have several common characteristics that distinguish that species from

Figure 9.1 Carolus Linnaeus (1707–1778). Linnaeus is known as the father of taxonomy. He is shown here in the cross-country skiing outfit he wore to collect specimens in Lapland. The curled boot toes held his skis onto his feet.

TABLE 9.1

The Meaning of the Names of Some Microorganisms

Name of Microorganism	Meaning of Name
Entamoeba histolytica	*Ent*, intestinal; *amoebae*, shape and means of movement; *histo*, tissue; *lytic*, lysing, or digesting tissue
Escherichia coli	Named after Theodor Escherich in 1888; found in the colon
Haemophilus ducreyi	*Hemo*, blood; *phil*, love; named after Augusto Ducrey in 1889
Neisseria gonorrhoeae	Named after Albert L. Neisser in 1879; causes gonorrhea
Saccharomyces cerevisiae	*Saccharo*, sugar; *myco*, mold; *cerevisia*, beer or ale
Staphylococcus aureus	*Staphylo*, cluster; *kokkus*, berry; *aureus*, golden
Lactococcus lactis	*Lacto*, milk; *kokkus*, berry
Shigella etousae	Named after Kiyoshi Shiga in 1898; European Theater of Operations of the U.S. Army (final *e* gives proper Latin ending)

all other species. As a rule, members of the species cannot be divided into significantly different groups on the basis of a particular characteristic, but there are exceptions to this rule. Sometimes members of a species are divided on the basis of a small but permanent genetic difference, such as a need for a particular nutrient, resistance to a certain antibiotic, or the presence of a particular antigen. When organisms in one pure culture of a species differ from the organisms in another pure culture of the same species, the organisms in each culture are designated as strains. A **strain** is a subgroup of a species with one or more characteristics that distinguish it from other subgroups of the same species. Each strain is identified by a name, number, or letter that follows the specific epithet. For example, *E. coli* strain K12 has been extensively studied because of its plasmids and other genetic characteristics, and *E. coli* strain 0157:H7 causes hemorrhagic inflammation of the colon in humans.

About 4,400 animals, and over 7,700 plants still retain the names Linnaeus gave them. An "L." after a species name indicates Linnaeus named it.

In addition to introducing the binomial system of nomenclature, Linnaeus also established a hierarchy of taxonomic ranks: species, genus, family, order, class, phylum or division, and kingdom. At the highest level, Linnaeus divided all living things into two *kingdoms*—plant and animal. In his taxonomic hierarchy, much of which is still used today, each organism is assigned a species name, and species of very similar organisms are grouped into a genus. As we proceed up the hierarchy, several similar genera are grouped to form a *family*, several families to form an *order*, and so on to the top of the hierarchy. Some hierarchies today have additional levels, such as *subphyla*. Also, it has become accepted practice to refer to the first categories within the animal kingdom as *phyla* and to those within other kingdoms (we now have five) as *divisions*. Recently, the five kingdoms have been grouped together into three *domains*, a new category even higher than kingdom. Domains will be discussed later in the chapter. The classifications of a human, a dog, a wolf, and a bacterium are shown in Figure 9.2.

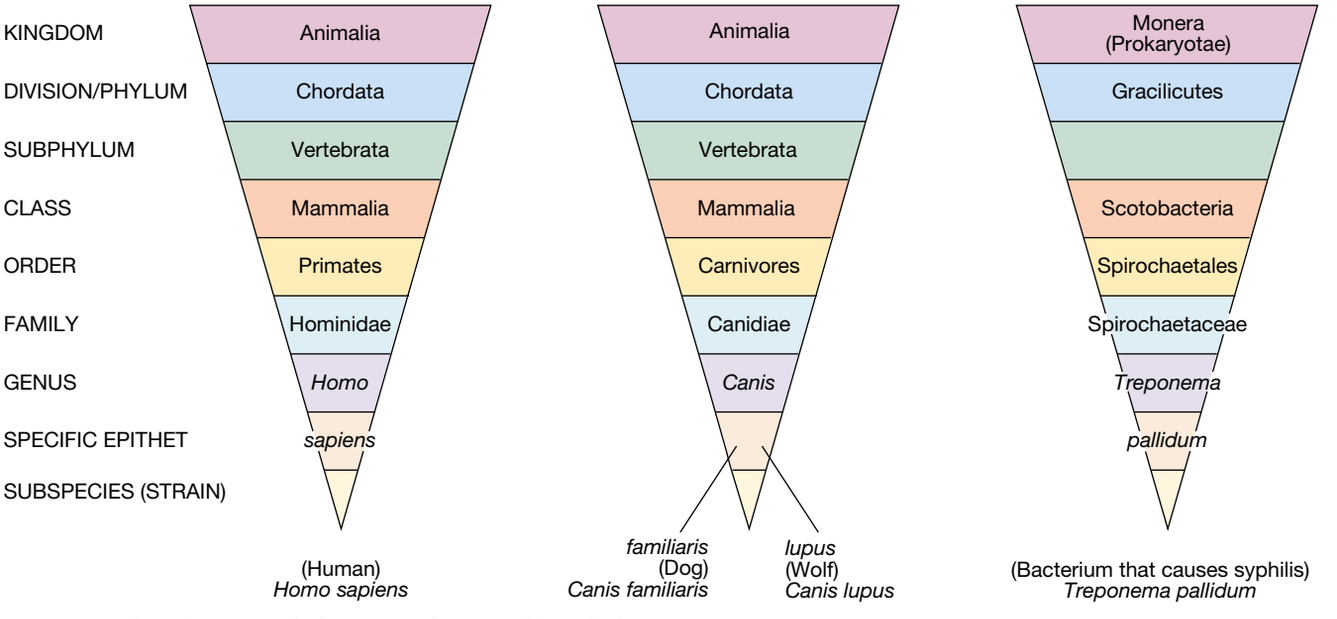


Figure 9.2 **Classification of a human, a dog, a wolf, and a bacterium.**

USING A TAXONOMIC KEY

Biologists often use a taxonomic *key* to identify organisms according to their characteristics. The most common kind is a **dichotomous key**, which has paired statements describing characteristics of organisms. Paired statements present an "either-or" choice, so that only one statement is true. Each statement is followed by directions to go to another pair of statements until the name of the organism finally appears. Figure 9.3 is a dichotomous key that will identify each of the four most common U.S. coins: quarters, dimes, nickels, and pennies. Read statements 1a and 1b, and decide which statement applies to a given coin. Look at the number to the right of the statement; it tells you which pair of statements to look at next. Continue in this manner until you reach a group designation. If you have followed the key carefully, that designation will name the coin.

Of course, you don't need a taxonomic key to identify something as simple and as familiar as coins. But identifying all the many kinds of bacteria in the world is a more difficult task. Major groups of bacteria can be identified with the key in Figure 9.4. More detailed keys use staining reactions, metabolic reactions (fermentation of particular sugars or release of different gases), growth at different temperatures, properties of colonies on solid media, and similar characteristics of cultures. By proceeding step by step through the key, one should be able to identify an unknown organism, or even a strain, if the key is sufficiently detailed.

Problems in Taxonomy

Among the aims of a taxonomic system are organizing knowledge about living things and establishing standard names for organisms so that we can communicate about them. Ideally, we would like to classify organisms according to their **phylogenetic**, or evolutionary, relationships, but this is not always easy. Evolution occurs continuously and at a relatively rapid rate in microorganisms, and our knowledge of the evolutionary history of organisms is incomplete. Taxonomy must change with evolutionary changes and new knowledge. *It is far more important to have a taxonomic system that reflects our current knowledge than to have a system that never changes.*

1a	Smooth-edged	Go to 2
1b	Rough-edged	Go to 3
2a	Silver-colored	Nickel
2b	Copper-colored	Penny
3a	Large (about 1-in. diameter)	Quarter
3b	Small (about 3/4-in. diameter)	Dime

Figure 9.3 A dichotomous key for classifying typical U.S. coins. Why would the word "*flat*" not be useful in this key?

1a	Gram-positive	Go to 2
1b	Not Gram-positive	Go to 3
2a	Cells spherical in shape	Gram-positive cocci
2b	Cells not spherical in shape	Go to 4
3a	Gram-negative	Go to 5
3b	Not Gram-negative (lack cell wall)	Mycoplasma
4a	Cells rod-shaped	Gram-positive bacilli
4b	Cells not rod-shaped	Go to 6
5a	Cells spherical in shape	Gram-negative cocci
5b	Cells not spherical in shape	Go to 7
6a	Cells club-shaped	Corynebacteria
6b	Cells variable in shape	Propionibacteria
7a	Cells rod-shaped	Gram-negative bacilli
7b	Cells not rod-shaped	Go to 8
8a	Cells helical with several turns	Spirochetes
8b	Cells comma-shaped	Vibrioids

Figure 9.4 A dichotomous key for classifying major groups of bacteria.

Creating a taxonomic system that provides an organized overview of all living things and how they are related

CLOSE-UP

Mail-Order Microbes

Do you wonder when you sip wine or eat cheese, where the microorganisms that made those products come from? They might be from the American Type Culture Collection (ATCC) in Manassas, Virginia, which keeps some preserved cultures in a secured vault to protect them against theft. Yes, theft—some strains of organisms are valuable enough to be kept here because their characteristics are important in research or in industrial applications such as winemaking. Many organisms are preserved in a dormant state to prevent them from undergoing genetic changes that might alter their characteristics. Different researchers can order particular strains from the ATCC, ensuring that all tests done anywhere around the world with these mail-ordered organisms are using genetically identical microbes. Manufacturers of wine or cheese can be sure that they will always be able to obtain the organisms that make products with particular distinctive flavors or other characteristics.

to each other poses certain problems. Two such problems arise at opposite ends of the taxonomic hierarchy: (1) deciding what constitutes a species, and (2) deciding what constitutes a kingdom or in which domain a kingdom belongs. In the first case, taxonomists try to decide how much diversity can be tolerated within the unity of a species. In the second, taxonomists try to decide how to sort the diverse characteristics of living things into categories that reflect fundamental differences of evolutionary significance. In most advanced organisms, such as plants and animals, species that reproduce sexually are distinguished primarily by their reproductive capabilities. A male and a female of the same species are capable of DNA transfer through mating and producing fertile offspring, whereas members of different species ordinarily either cannot mate successfully or will have sterile offspring. *Morphology* (structural characteristics) and geographic distribution also are considered in defining species.

In bacteria, such criteria normally cannot be used in defining a species, primarily because lateral gene transfer (genetic recombination) among bacteria has been very common in evolution, but morphological differences are minor. A bacterial species is defined by the similarities found among its members. Properties such as biochemical reactions, chemical composition, cellular structures, genetic characteristics, and immunological features are used in defining a bacterial species. Identifying a species and determining its limits present the most challenging aspects of biological classification—for any type of organism.

Before taxonomists turned their attention to microorganisms, the two-kingdom system of plants and animals worked reasonably well. Anyone can tell plants from animals—for example, trees from dogs. Plants make their own food but cannot move, and animals move but cannot make their own food. Simple enough, or is it? In this scheme, how do you classify *Euglena*, a mobile microorganism that makes its own food? How would you classify jellyfishes and sponges, which are motile or immotile depending on their stage of life? And how do you classify colorless fungi that neither move nor make their own food? Finally, how do you classify slime molds, organisms that can be unicellular or multicellular and mobile or immobile? Obviously, many organisms pose a number of problems when one tries to use a two-kingdom system.

Developments Since Linnaeus's Time

The problem of classifying microorganisms was first addressed by the German biologist Ernst H. Haeckel in 1866 when he created a third kingdom, the Protista. He included among the protists all "simple" forms of life such as bacteria, many algae, protozoa, and multicellular fungi and sponges. Haeckel's original term, Protista, is still used in taxonomic schemes today, but it is now limited mainly to unicellular eukaryotic organisms.

Classification of bacteria has posed taxonomic problems over the centuries and still does. Until recently, many taxonomists regarded bacteria as small plants that lacked chlorophyll. As late as 1957, the seventh edition of *Bergey's Manual of Determinative Bacteriology*, a work devoted to the identification of bacteria, considered bacteria to be unicellular plants. Changes in this viewpoint came as the tools to study bacteria were developed. First, light microscopy and staining techniques were used to describe the basic structure of cells. Second, electron microscopy was used to study the ultrastructure of cells. And third, biochemical techniques were used to study chemical composition and chemical reactions in cells. One of the most important discoveries from these various studies was that DNA looked and behaved differently during cell division in bacteria than in cells whose DNA is organized into chromosomes within a nucleus.

Studies of the structure and function of cells also led to the recognition of two general patterns of cellular organization, prokaryotic and eukaryotic. Basing taxonomy on these two different patterns of cellular organization was proposed as early as 1937. Various taxonomists such as H. F. Copeland, R. Y. Stanier, C. B. van Niel, and R. H. Whittaker, working in the late 1950s, placed bacteria in a separate kingdom of anucleate (lacking a cell nucleus) organisms rather than with organisms that have true nuclei. In 1962, Stanier and van Niel stated, "The distinctive property of bacteria is the prokaryotic nature of their cells."

In 1956, Lynn Margulis and H. F. Copeland proposed a scheme of classifying prokaryotes and eukaryotes by the following four-kingdom system of classification:

1. Monera: all prokaryotes, including true bacteria and blue-green algae.
2. Protoctista: all eukaryotic algae, protozoa, and fungi.
3. Plantae: all green plants.
4. Animalia: all animals derived from a zygote, a cell formed by the union of an egg and a sperm.

These taxonomists also proposed that evolution from prokaryotic to eukaryotic life forms had taken place by endosymbiosis (◀Chapter 4, p. 101).

R. H. Whittaker felt that endosymbiosis could not account for all the differences between prokaryotes and eukaryotes. He also felt that a taxonomic system should give more consideration to the methods organisms use to obtain nourishment. Autotrophic nutrition by photosynthesis and heterotrophic nutrition by the ingestion of substances from other organisms had been considered in earlier taxonomies. Absorption as a sole means of acquiring nutrients had been overlooked. To Whittaker, fungi, which acquire nutrients solely by absorption, were sufficiently different from plants to justify placing them in a different kingdom. Also, fungi have certain reproductive processes not shared with any other organisms. Consequently, Whittaker proposed a taxonomic system in 1969 that separated the Protoctista into two kingdoms—Protista (pro-tis′tah) and Fungi—but retained the Monera,

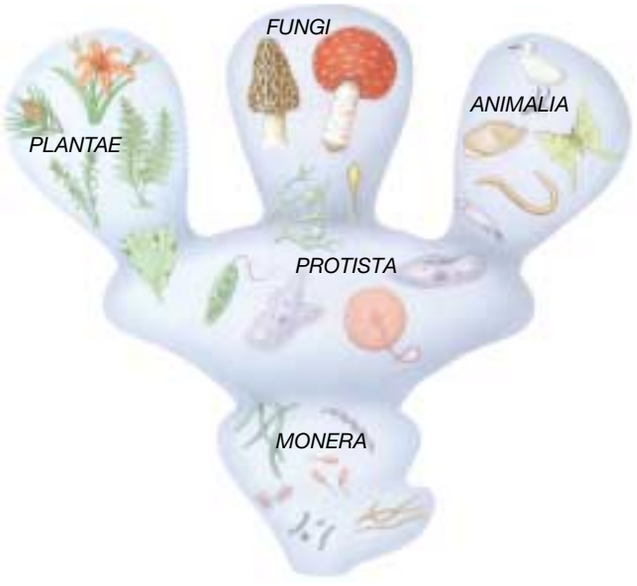


Figure 9.5 **The five-kingdom system of classification.**

Plantae, and Animalia. Finally, through refinements of Whittaker's system by several taxonomists over the past few decades, the five-kingdom system was created.

THE FIVE-KINGDOM CLASSIFICATION SYSTEM

Before we discuss the five-kingdom classification system and how it applies to microorganisms, we must emphasize that all living organisms, regardless of the kingdom to which they are assigned, display certain characteristics that define the unity of life. All organisms are composed of cells, and all carry out certain functions, such as obtaining nutrients and getting rid of wastes. The cell is the basic structural and functional unit of all living things. The fact that viruses are not cells is one reason they are not considered to be living organisms. All cells are bounded by a cell or plasma membrane, carry genetic information in DNA, and have ribosomes where proteins are made. All cells also contain the same kinds of organic compounds—proteins, lipids, nucleic acids, and carbohydrates. They also selectively transport material between their cytoplasm and their environment. Thus, although organisms may be classified in very diverse taxonomic groups, their cells have many similarities in structure and function.

No single classification system is completely accepted by all biologists. One of the most widely accepted is the **five-kingdom system** (Figure 9.5). A major advantage of this system is the clarity with which it deals with microorganisms. It places all **prokaryotes**, microorganisms that lack a cell nucleus, in the kingdom Monera (Prokaryotae) (◀Chapter 4, p. 77). It places most unicellular **eukaryotes**, organisms whose cells contain a distinct nucleus, in the kingdom Protista. (Margulis proposed a very similar five-kingdom system in 1982, but she referred to the kingdom of simple eukaryotes as Protoctista instead of Protista.) The five-kingdom system also places fungi in the separate kingdom Fungi.

The properties and members of each of the five kingdoms are described below and summarized in Table 9.2. A more detailed classification of bacteria is provided in Appendix B.

Kingdom Monera

The kingdom **Monera** (mo-ner′ah) is also called the kingdom **Prokaryotae**, as suggested by the French marine biol-

TABLE 9.2

The Five-Kingdom System of Classification

	Monera (Prokaryotae)	Protista	Fungi	Plantae	Animalia
Cell type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Cell organization	Unicellular; occasionally grouped	Unicellular; occasionally multicellular	Unicellular or multicellular	Multicellular	Multicellular
Cell wall	Present in most	Present in some, absent in others	Present	Present	Absent
Nutrition	Absorption, some photosynthetic, some chemosynthetic	Ingestion or absorption, some photosynthetic	Absorption	Absorptive, photosynthetic	Ingestion; occasionally in some parasites by absorption
Reproduction	Asexual, usually by binary fission	Mostly asexual, occasionally both sexual and asexual	Both sexual and asexual, often involving a complex life cycle	Both sexual and asexual	Primarily sexual

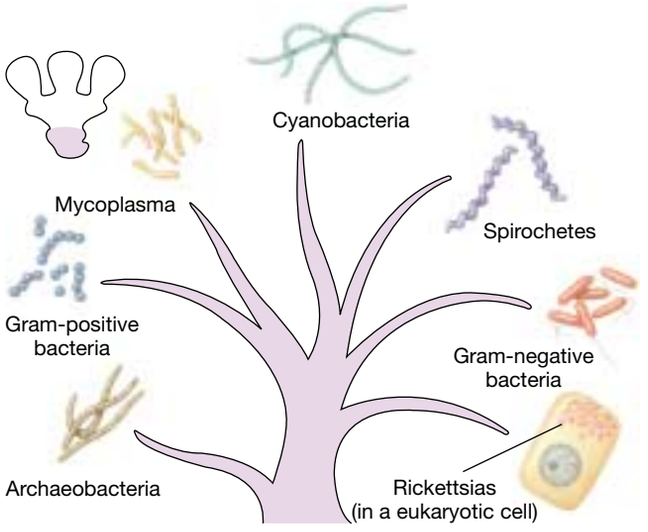


Figure 9.6 Some typical monerans. Monerans are prokaryotic organisms without a cell nucleus and other internal, membrane-enclosed structures.

CLOSE-UP

Going Where None Have Gone Before

If you were a microorganism, you'd love to be able to grow in places where competing microorganisms couldn't survive. That's what the archaeobacteria have going for them. At the time of their discovery in 1977, the archaeobacteria were already thought to be quite odd. They lived in brines five times as salty as the oceans, geothermal environments that would cook other organisms to a crisp, and anaerobic habitats where even trace amounts of oxygen couldn't be found. Now they have been proven to be even odder. *Pyrolobus fumarii* holds the current record for life at high temperatures, growing at temperatures as high as a scalding 113°C. Antarctic archaeobacteria thrive at −1.8°C. Archaeobacteria have also been found in rice paddies, terrestrial soils, freshwater lake sediments, and even winery by-products.

ogist Edouard Chatton in 1937. It consists of all prokaryotic organisms, including the eubacteria ("true bacteria"), the cyanobacteria, and the archaeobacteria (Figure 9.6).

All monerans are unicellular; they lack true nuclei and generally lack membrane-enclosed organelles. Their DNA has little or no protein associated with it. Reproduction in the kingdom Monera occurs mainly by binary fission. Of all monerans, the **eubacteria** (u′bak-ter″e-ah) are of greatest concern in the health sciences and will be considered in detail in several chapters of this book.

The **cyanobacteria** (si″an-o-bak-ter′e-ah), formerly known as blue-green algae, are of special importance in the balance of nature. They are photosynthetic, typically unicellular organisms, although cells may sometimes be connected to form threadlike filaments. Being autotrophs, cyanobacteria do not invade other organisms, so they pose no health threat to humans, except for toxins (poisons) some release into water.

Cyanobacteria grow in a great variety of habitats, including anaerobic ones, where they often serve as food sources for more complex heterotrophic organisms. Some "fix" atmospheric nitrogen, converting it to nitrogenous compounds that algae and other organisms can use. Certain cyanobacteria also thrive in nutrient-rich water and are responsible for algal blooms—a thick layer of algae on the surface of water that prevents light from penetrating to the water below. Such blooms release toxic substances that can give the water an objectionable odor and even harm fish and livestock that drink the water.

Archaeobacteria (ar′ke-o-bak′ter″e-ah) surviving today are primitive prokaryotes adapted to extreme environments. The methanogens reduce carbon-containing compounds to the gas methane. The extreme halophiles live in excessively salty environments, and the thermoacidophiles live in hot acidic environments, such as volcanic vents in the ocean floor (Figure 9.7). Believed to be of very ancient origin, archaeobacteria have been found to differ from eubacteria in several distinctive ways, including the structure of their cell wall and the structure of their RNA polymerase. These organisms will be discussed in greater detail later in the chapter.

Figure 9.7 Archaeobacteria: extremophiles that can exploit the unusual habitat of a "black smoker" vent. Living at deep ocean vents, where hot sulfurous volcanic gases are released from the Earth's interior, archaeobacteria survive in one of the most extreme environments known. This vent is located in the Mid-Atlantic Ridge, 3,100 meters below sea level—beneath a tremendous pressure of water, and at a temperature of 360°C. The bacteria obtain their energy from the sulfur compounds.

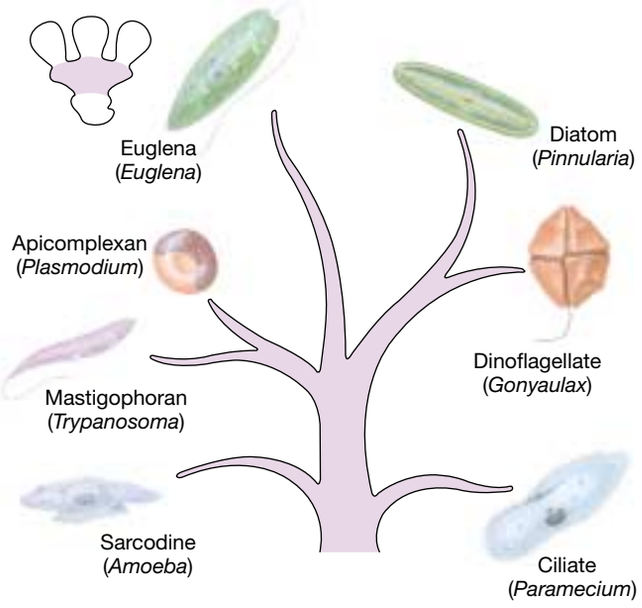


Figure 9.8 Some typical protists. Protists are unicellular, eukaryotic organisms.

Kingdom Protista

Although the modern protist group is very diverse, it contains fewer kinds of organisms than when first defined by Haeckel. All organisms now classified in the kingdom **Protista** (Figure 9.8) are eukaryotic. Most are unicellular, but some are organized into colonies. Protists have a true membrane-enclosed nucleus and organelles within their cytoplasm, as do other eukaryotes. Many protists live in fresh water, some live in seawater, and a few live in soil. They are distinguished more by what they don't have or don't do than by what they have or do. Protists do not develop from an embryo, as plants and animals do, and they do not develop from distinctive spores, as fungi do. Yet, among the protists are the algae, which resemble plants; the protozoa, which resemble animals; and the euglenoids, which have both plant and animal characteristics. The protists of greatest interest to health scientists are the protozoa that can cause disease (◀Chapter 11, p. 305).

Kingdom Fungi

The kingdom **Fungi** (Figure 9.9) includes mostly multicellular and some unicellular organisms. Fungi obtain nutrients solely by absorption of organic matter from dead organisms. Even when they invade living tissues, fungi typically kill cells and then absorb nutrients from them. Although the fungi have some characteristics in common with plants, their structures are much simpler in organization than true leaves or stems. Fungi form spores but do not form seeds. Many fungi pose no threat to other living things, but some attack plants and animals, even humans (◀Chapter 11, p. 308). Others such as yeast and mushrooms are important as foods or in food production (◀Chapter 26, p. 791).

Kingdom Plantae

The placement of most microscopic eukaryotes with the protists leaves only macroscopic green plants in the kingdom **Plantae**. Most plants live on land and contain chlorophyll in organelles called chloroplasts. Plants are of interest to microbiologists because some contain medicinal substances such as quinine, which has been used to treat microbial infections. Many microbiologists are very interested in plant-microbe interactions, particularly with regard to plant pathogens, which threaten food supplies.

Kingdom Animalia

The kingdom **Animalia** includes all animals derived from zygotes (a cell formed by the union of two gametes, such as an egg and a sperm). Although nearly all members of this kingdom are macroscopic and therefore of no concern to microbiologists, several groups of animals live in or on other organisms, and some serve as carriers of microorganisms (Figure 9.10).

Certain *helminths* (worms) are parasitic in humans and other animals. Helminths include flukes, tapeworms, and roundworms, which live inside the body of their host. They also include leeches, which live on the surface of their hosts. Microbiologists often need to identify both microscopic and macroscopic forms of helminths (◀Chapter 11, p. 314).

Helminthiasis is the most widespread human parasitic infection. Ascaris now infects 1.4 billion people; Trichuris, 1.3 billion; and hookworms, 2 billion.

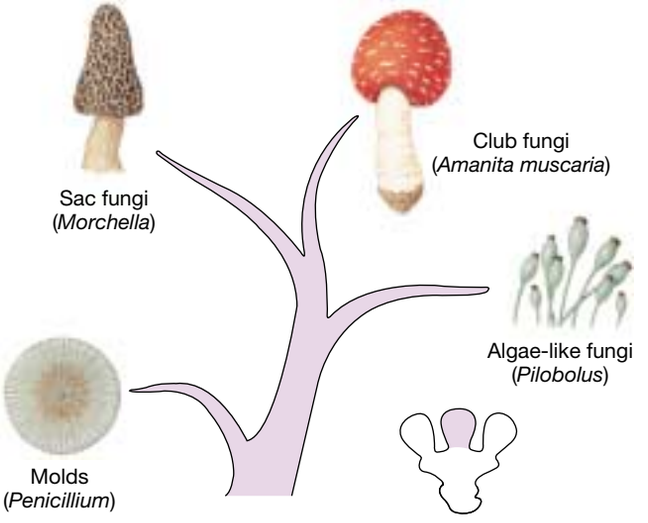


Figure 9.9 Some typical fungi. Fungi are eukaryotic organisms that have cell walls and do not carry out photosynthesis. Fungi take their food from other organic sources (that is, they are chemoheterotrophs).

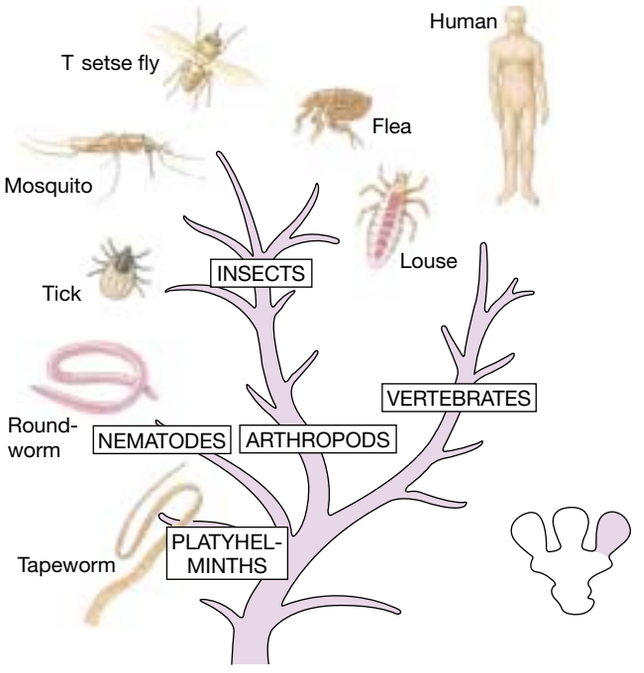


Figure 9.10 **Groups from the kingdom Animalia that are relevant to microbiology.**

Certain *arthropods* live on the surface of their hosts, and some spread disease. Ticks, mites, lice, and fleas are arthropods that live on their hosts for at least part of their lives. Ticks, lice, fleas, and mosquitoes can spread infectious microorganisms from their bodies to those of humans or other animals (◀Chapter 11, p. 321).

THE THREE-DOMAIN CLASSIFICATION SYSTEM

Studies of the archaeobacteria in the late 1970s by Carl Woese, G. E. Fox, and others suggested that these organisms represent a third cell type, and they proposed another scheme for the evolution of living things from a universal common ancestor (Figure 9.11). They hypothesized that a group of *urkaryotes*, the earliest or original cells, gave rise to the eukaryotes directly rather than by way of prokaryotes. They proposed that nucleated urkaryotes became true eukaryotes by acquiring organelles by endosymbiosis from certain eubacteria.

The Evolution of Prokaryotic Organisms

At about the same time that archaeobacteria were first being investigated, studies of stromatolites were also being conducted. **Stromatolites** (stro-mat′o-lites) are fossilized photosynthetic prokaryotes that appear as masses of cells or microbial mats. Commonly found associated with lagoons or hot springs, they are still forming today. Because stromatolites are fossilized prokaryotes, they do not provide any evidence for phylogenetic, or evolutionary, relationships but can be used to determine the period during which they arose. Studies of stromatolites indicate that life arose nearly 4 billion years ago, and that an "Age of Microorganisms," in which there were no multicellular living organisms, lasted for about 3 billion years. Combined evidence from studies of archaeobacteria and the most ancient stromatolites convinced many scientists that three branches of the tree of life formed during the Age of Microorganisms, and that each branch gave rise to distinctly different groups of organisms.

The three-branch tree was not accepted by all scientists. In 1977, the English taxonomist T. Cavalier-Smith proposed instead that the archaeobacteria arose later than the eubacteria by divergent evolution from a group of Gram-positive bacteria similar to present-day actinomycetes, which were once thought to be fungi. The American taxonomist J. A. Lake proposed in 1988 still another model with two main branches. In Lake's model, one branch gave rise to the eubacteria and to two groups of archaeobacteria—those that live in extremely salty environments and those that release methane. The other branch gave rise to the eukaryotes and to a group of archaeobacteria that Lake calls the *eocytes*, which grow in hot, acidic environments.

Fossilized stromatolites are so common in China that they are used for flooring and as a surface for childrens' playground slides.

In 1990, Woese suggested that a new taxonomic category, the **domain**, be erected above the level of kingdom. He based this suggestion on comparative studies of prokaryotes and eukaryotes at the molecular level and of their probable evolutionary relationships. Woese concluded that the archaeobacteria may be more closely related to eukaryotes than to eubacteria.

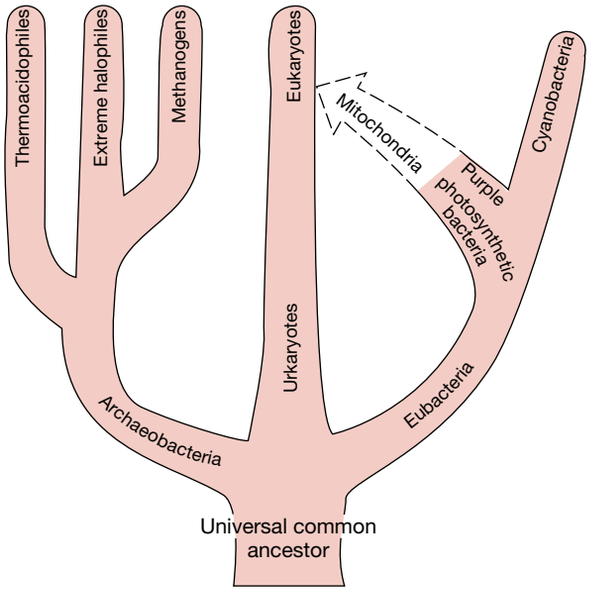


Figure 9.11 **A model of the major evolutionary lines of descent proposed after the discovery of archaeobacteria.**

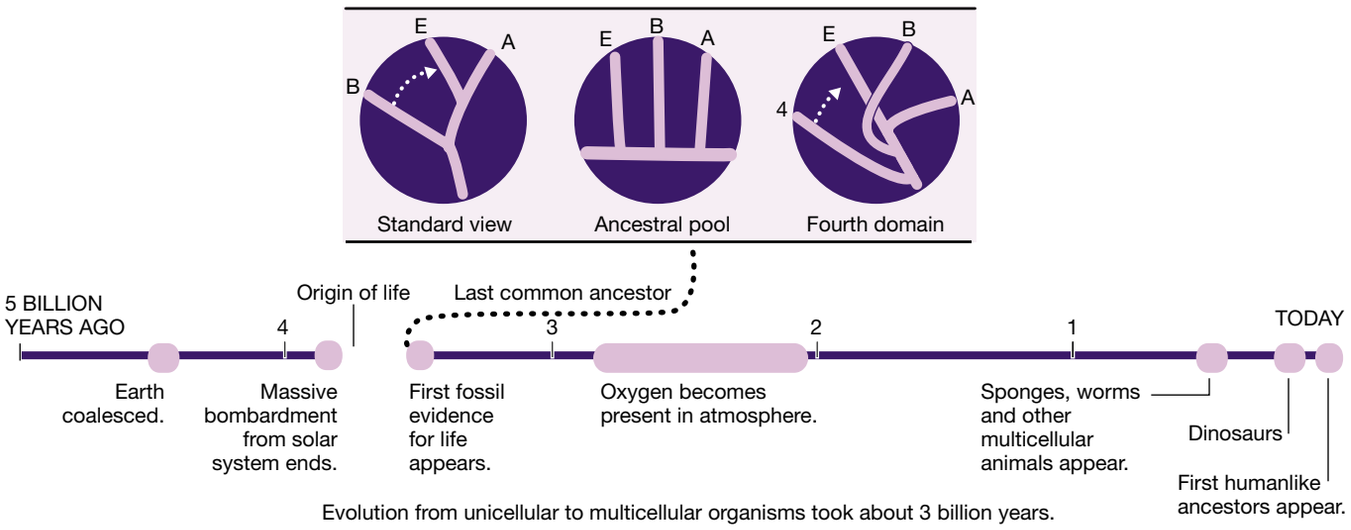


Figure 9.12 **Theories about the three domains.** The standard view is that the universal ancestor split into Bacteria and Archaea, and the Eukarya then branched off from the Archaea. An emerging view is that all three branches evolved independently from the same pool of genes. A third view is that there was a fourth branch, now lost, that contributed genes to the Eukarya. (*Source:* Adapted from Dr. Carl Woese and Dr. Norman R. Pace, *New York Times,* April 14, 1998, p. C1.)

In 1998, Woese discussed theories about how the three domains may have arisen (Figure 9.12). The standard view was that a universal common ancestor first split into **Bacteria** and **Archaea**, and then the **Eukarya** branched off from Archaea. A second view held that all three domains arose simultaneously from a pool of common ancestors that were all able to exchange genes with one another—hence, the universal genetic code. A third view sought to explain how so many genes are present in Eukarya but lacking in Archaea and Bacteria. It postulated the existence of a fourth domain that directly contributed genes to the Eukarya and then became extinct. Thus, we see no modern-day descendants of this group.

The three domains Woese proposed are shown in Figure 9.13. The domain Eukarya contains all those kingdoms of eukaryotic organisms—the animals, plants, fungi, and protists. The traditional kingdom Monera has been divided into two domains: the domain Bacteria and the domain Archaea. A comparison of the three domains is presented in Table 9.3.

TABLE 9.3

Bacteria, Archaea, and Eukarya Compared

	Bacteria	Archaea	Eukarya
Cell type	Prokaryotic	Prokaryotic	Eukaryotic
Typical size	0.5–4 μm	0.5–4 μm	>5 μm
Cell wall	Usually present, contain peptidoglycan	Present, lack peptidoglycan	Absent or made of other materials
Lipids in membranes	Fatty acids present, linked by ester bonds	Isoprenes present, linked by ester bonds	Fatty acids present, linked by ester bonds
Protein synthesis	First amino acid = methionine; impaired by antibiotics such as chloramphenicol	First amino acid = formylmethionine; not impaired by antibiotics such as chloramphenicol	First amino acid = methionine; most not impaired by antibiotics such as chloramphenicol
Genetic material	Small circular chromosome and plasmids; histones absent	Small circular chromosome and plasmids, histonelike proteins present	Complex nucleus with more than one large, linear chromosome, histones present
RNA polymerase	Simple	Complex	Complex
Locomotion	Simple flagella, gliding, gas vesicles	Simple flagella, gas vesicles	Complex flagella, cilia, legs, fins, wings
Habitat	Wide range of environments	Usually only extreme environments	Wide range of environments
Typical organisms	Enteric bacteria, cyanobacteria	Methane-producing bacteria, halobacteria, extreme thermophiles	Algae, protozoa, fungi, plants, and animals

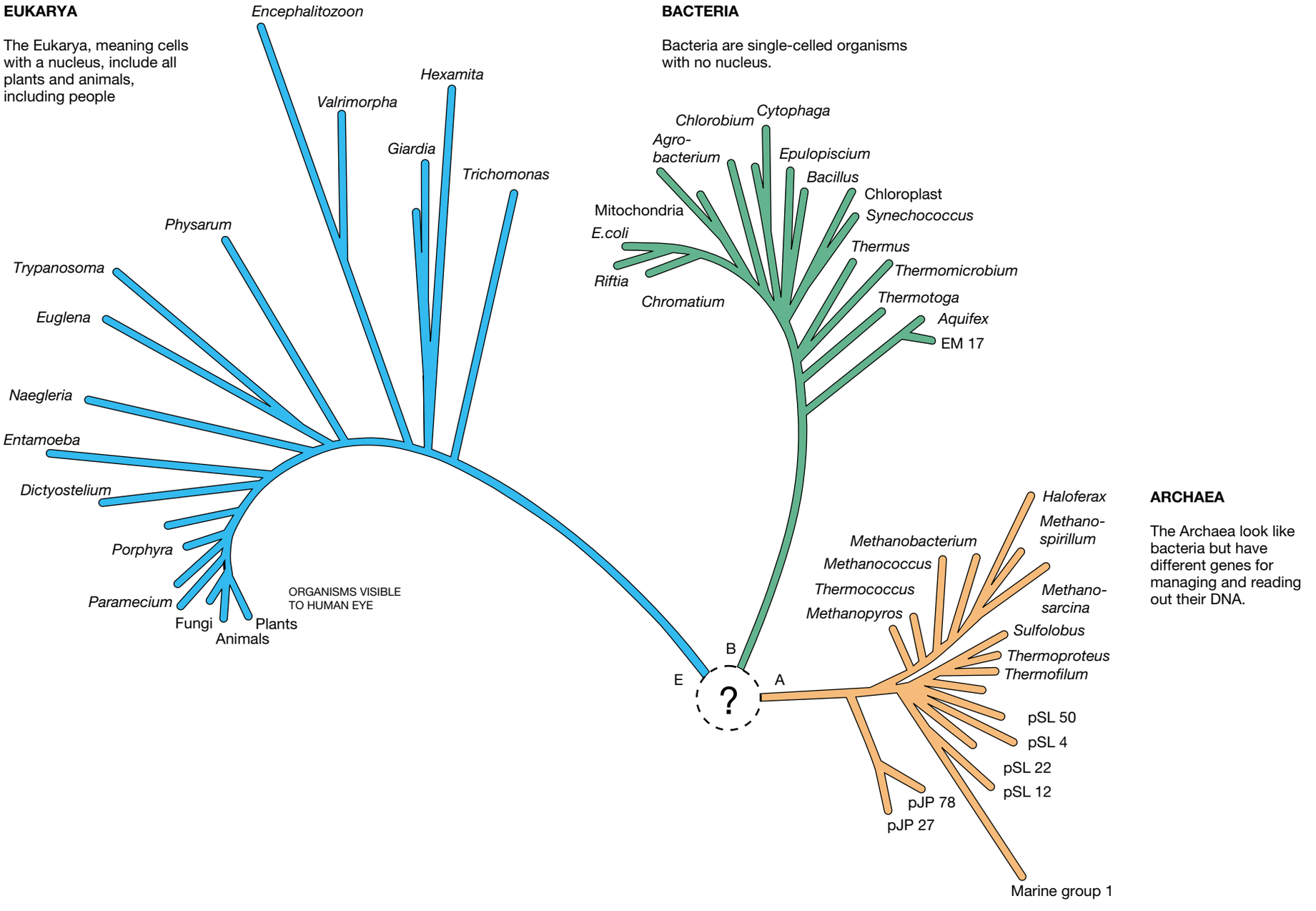


Figure 9.13 **The three-domain system of classification.** Shown here are selected members of the three domains. Lengths of the branches indicate the extent of genetic differences in each organism, based on the similarities of their ribosomal RNA. (*Source:* Adapted from Dr. Carl Woese and Dr. Norman R. Pace, *New York Times*, April 14, 1998, p. C1.)

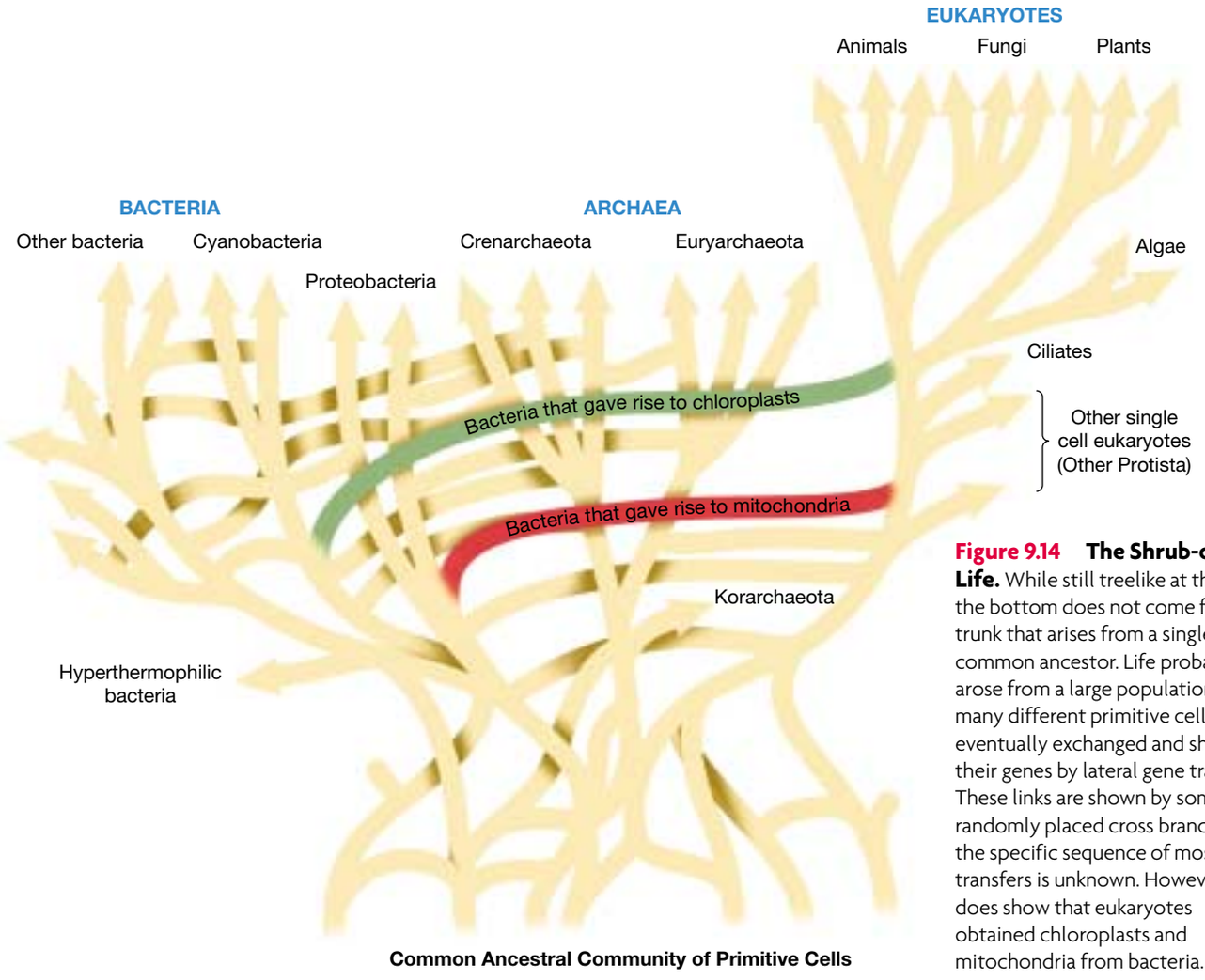


Figure 9.14 The Shrub-of-Life. While still treelike at the top, the bottom does not come from a trunk that arises from a single common ancestor. Life probably arose from a large population of many different primitive cells that eventually exchanged and shared their genes by lateral gene transfer. These links are shown by somewhat randomly placed cross branches, as the specific sequence of most transfers is unknown. However, it does show that eukaryotes obtained chloroplasts and mitochondria from bacteria.

The Tree of Life is Replaced by a Shrub

As complete sequences of genomes are becoming available in increasing numbers, the concept of a *universal common ancestor* giving rise to a linear, branching tree of life is now seen as oversimplified, or just plain wrong! According to the standard view (Figure 9.12), the common ancestral line first broke into two lines: the Bacteria and the Archaea. The Eukarya branch then split off from the Archaea and later received genes twice from Bacteria: once for chloroplasts (and photosynthesis) and once for mitochondria (and respiration). Thus, Archaea should have no Bacterial genes, and Eukarya should have only those dealing with photosynthesis and respiration. However—this is *not* the way things are! *Thermotoga maritima*, the Bacterium sequenced by Karen Nelson, has 24% of its genome made up of archaeal genes, which she believes were acquired by lateral gene transfer (◀Chapter 8, p. 205). The Archaean, *Archaeoglobus fulgidus*, has numerous Bacterial genes that help it utilize undersea oils. And many Eukarya have Bacterial genes that have nothing to do with photosynthesis or respiration. Some organisms have genes from all three domains. W. Ford Doolittle of Dalhousie University in Nova Scotia, Canada, has come up with a "**shrub of life**" diagram that better represents our current understanding of the early evolution of life (Figure 9.14). There are many roots, rather than a single ancestral line, and the branches crisscross and merge again and again. The mergings do not represent joinings of entire genomes, but only transfers of a single or a few genes (Figure 9.15).

We know that lateral gene transfer, that is, gene swapping with contemporary organisms, occurs today. This is how antibiotic resistance genes, transported by plasmids, are spread among various bacteria. What we are only just now beginning to learn is how important a force in evolution lateral gene transfer has been and continues to be. Does this all seem confusing? Have you been led down a wrong road? Doolittle answers,

> Some biologists find these notions confusing and discouraging. It is as if we have failed at the task Darwin set for us: delineating the unique structure of the tree of life. But in fact, our science is working just as it should. An attractive

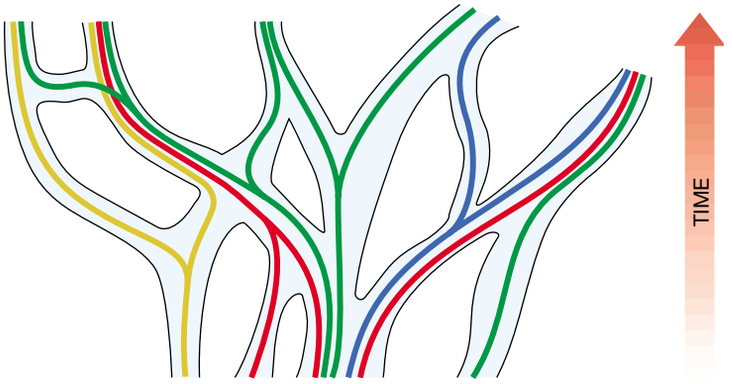


Figure 9.15 **Lateral gene transfer.** Colored lines, arising from an assortment of different ancestral cells, indicate lateral transfer of genes from one cell type to another. As genes from varying sources are combined, they give rise to new types of cell lines that have multiple origins of ancestry.

> hypothesis or model (the single tree) suggested experiments, in this case the collection of gene sequences and their analysis with the methods of molecular phylogeny. The data show the model to be too simple. Now new hypotheses, having final forms we cannot yet guess, are called for.
>
> W. Ford Doolittle, "Uprooting the Tree of Life," *Scientific American* (February 2000), p. 95.

The Archaea

The Archaea exhibit many differences from the Bacteria. One of the first variations to be noted was that of cell wall structure, and thus far a significant number of variations have been observed (see Table 9.3). However, not all archaeobacteria are the same. Three major groups of archaeobacteria are commonly recognized: methanogens, extreme halophiles, and extreme thermophiles. These groupings are based on physiological characteristics of the organisms and therefore cannot be considered phylogenetic, or evolutionary, classifications. The **methanogens** are strictly anaerobic organisms, having been isolated from such divergent anaerobic environments as waterlogged soils, lake sediments, marshes, marine sediments, and the gastrointestinal tracts of animals, including humans. As members of the anaerobic food chain, they degrade organic molecules to methane. **Extreme halophiles** grow in highly saline environments such as the Great Salt Lake, the Dead Sea, salt evaporation ponds, and the surfaces of salt-preserved foods. Unlike the methanogens, extreme halophiles are generally obligate aerobes. The **extreme thermoacidophiles** occupy unique niches where bacteria are very rarely found, such as hot springs, geothermally heated marine sediments, and submarine hydrothermal vents. With optimum temperatures usually in excess of 80°C, they may be either obligate aerobes, facultative aerobes, or obligate anaerobes. The heat-stable enzymes known as *extremozymes* that are found in these organisms have become of special interest to scientists.

APPLICATION

The Uses of Extremozymes

Various archaeobacteria are able to survive under highly adverse environmental conditions—from freezing waters to deep-sea vents, from concentrated brine to hot sulfur springs. The conditions present in these environments would inactivate, or denature, most enzymes. In order for these organisms to not only survive but to even flourish under such conditions, they must possess special adaptations—namely, resistant enzymes. Enzymes that can survive and function under such adverse conditions are called *extremozymes*.

For many years, ordinary microbial enzymes have been used in manufacturing processes, such as the production of artificial sweeteners and "stonewashed" jeans, as well as in PCR and DNA fingerprinting. A big problem has been maintaining appropriate environmental conditions for the action or storage of microbial enzymes. The use of extremozymes would eliminate this concern. In PCR (◀Chapter 7, p. 196), the reactions must be cycled between low and high temperatures. The high temperature inactivates ordinary DNA polymerases, which then must be added again as the temperature lowers. The *Taq* DNA polymerase, isolated from the thermophile *Thermus aquaticus*, survives the high-temperature cycling and has enabled a totally automated PCR technology to be developed. An even more heat-resistant DNA polymerase, *Pfu*, has been isolated from the hyperthermophile *Pyrococcus furiosus* ("flaming fireball"). This enzyme works best at 100°C.

Proteases and lipases derived from alkaliphilic bacteria are being used as detergent additives to increase their stain-removal ability. They are also being used to produce the stonewashed appearance of denim. As more archaeobacteria and their extremoyzmes are discovered, new manufacturing applications are certain to be developed.

CLASSIFICATION OF VIRUSES

Viruses are acellular infectious agents that are smaller than cells. They contain nucleic acid (DNA or RNA) and are coated with protein. They have not been assigned to a kingdom. In fact, they display only a few characteristics associated with living organisms.

Initially viruses were classified according to the hosts they invaded and by the diseases they caused. As more was learned about viruses, the early concept of "one virus, one disease" used in classification was found to be invalid for many viruses. Today viruses

Who's responsible for naming viruses? This duty is performed by the over 400 participating virologists of the International Committee on Taxonomy of Viruses (ICTV).

CLOSE-UP

Viroids and Prions

Science changes. For a long time, everyone thought that viruses were the smallest infectious agents. But particles even smaller than viruses have recently been discovered, and some appear to serve as infectious agents. They include viroids (vir′oids) and prions (pre′onz). A viroid is simply a fragment of RNA. The viroid that causes potato spindle tuber disease contains only 359 bases—enough information to specify the location of only 119 amino acids if all the bases function as codons. That's one-tenth the amount of nucleic acid found in the smallest viruses! Prions, or *proteinaceous infections particles*, are only one-tenth the size of a virus and consist of a protein molecule that folds incorrectly as a result of mutation. These particles, which are self-replicating, are responsible for some mysterious brain infections in humans, as well as mad cow disease in cattle.

are classified by chemical and physical characteristics such as the type and arrangement of their nucleic acids, their shape (cubical or tubular), the symmetry of the protein coat that surrounds the nucleic acid, and the presence or absence of such things as a membrane covering (called an envelope), enzymes, tail structures, or lipids (Figure 9.16). These groupings reflect only common characteristics and are not intended to represent evolutionary relationships. A classification of viruses is presented in Appendix B.

The study of viruses, or *virology*, is extremely important in any microbiology course for two reasons: (1) Virology is a recognized branch of microbiology, and techniques to study viruses are derived from microbiological techniques; and (2) viruses are of concern to health scientists because many cause diseases in humans, other animals, plants, and even microorganisms.

✓ CHECKLIST

1. What is the difference between a taxon and taxonomy?
2. What is the difference between species and specific epithet?
3. What is meant by a system of taxonomy that is phylogenetic? Why does such a system change frequently?
4. What is the difference between a kingdom and a domain? Name the five kingdoms, the three domains, and the types of organisms contained in each.
5. Where do viruses, viroids, and prions fit in today's taxonomy?

THE SEARCH FOR EVOLUTIONARY RELATIONSHIPS

Many biologists are interested in how living things evolved and how they are related to one another. In fact, most people have some curiosity about how life originated and gave rise to the diverse assortment of living things we see today. Although the details of the search for evolutionary relationships are of interest mainly to taxonomists, they are of some significance to health scientists. For example, many of the biochemical properties used to establish evolutionary relationships also can be used in identifying microorganisms. Whether it be a symbiotic association (e.g., between nitrogen-fixed bacteria and legumes) or a relationship between an infectious agent and its host, evolutionary relationships generally evolve together. Knowledge of such evolution is useful in order to understand the circumstances under which one organism becomes capable of infecting another, sometimes resulting in a symbiotic relationship and other times in the disease process.

The shattering of the long-held belief that all bacteria have a single circular chromosome (Table 9.4) has raised many questions. For example, how did multiple chromosomes, some of which are linear, come into being? And lacking mitosis, how is it assured that each daughter cell will receive the correct number and kinds of chromosomes?

First, let us remember the definition of chromosome. Plasmids contain genes which are needed only occasionally and are not essential for continuous use. If a large plasmid (*megaplasmid*) acquires a collection of "housekeeping" genes which are needed for daily life, it is then elevated to the status of chromosome. Confusingly enough, genes and plasmids can be acquired either vertically or by horizontal transfer. And, transposons can relocate genes from chromosomes into plasmids. Or, a chromosome could break, releasing a self-replicating portion of its genome into the cytoplasm. These are all ways that an ancestor with a single chromosome can develop a second chromosome.

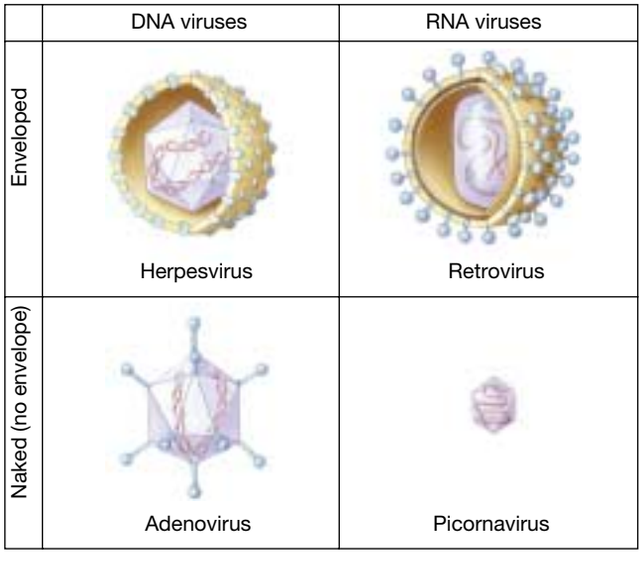


Figure 9.16 **Some categories of viruses.**

TABLE 9.4

Some Bacteria Having Two Chromosomes

Organism	Major Chromosome Size in Kilobases (kb) (1 kb = 1,000 bases)	Minor Chromosome Size in kb (1 kb = 1,000 bases)
Agrobacterium rhizogenes	4,000	2,700
Agrobacterium tumefaciens	3,000	2,100 (linear)
Rhizobium galegae	5,850	1,200
Rhizobium loti	5,500	1,200
Sinorhizobium meliloti	3,400	1,700
Brucella suis (biovar 3)	3,100	none
Brucella suis (biovar 2 and 4)	1,850	1,350
Brucella ovis	2,100	1,150
Brucella melitensis	2,100	1,150
Brucella abortus	2,100	1,150
Ochrobactrum intermedium	2,700	1,900
Rhodobacter sphaeroides	3,046	914
Deinococcus radiodurans	2,649	412

On the other hand, genomic studies of close bacterial relatives suggest that, in some cases, the ancestral organism had two chromosomes which eventually fused to become one. In fact, some units accepted uncritically as being plasmids, because of their small size, may actually contain essential genes and be small chromosomes. Some plasmidless species' genomes reveal plasmid-type virulence gene sequences located in their single chromosomes, most likely having arrived there by horizontal fusion.

Within separate strains (biovars) of a single species, such as *Brucella suis*, the genome may exist as one or as two chromosomes, without conferring any obvious advantage to either biovar. This implies that having one versus two chromosomes has no evolutionary impact, at least in this species. However, when duplicate genes are found on both of the two chromosomes within a cell, they may have slightly different products (due to mutations) which are regulated differently. This could be an advantage. Meanwhile, what do we call such cells? They are not haploid or monoploid, but are not fully diploid either, as only some genes are duplicated. The term *mesoploid* has been suggested.

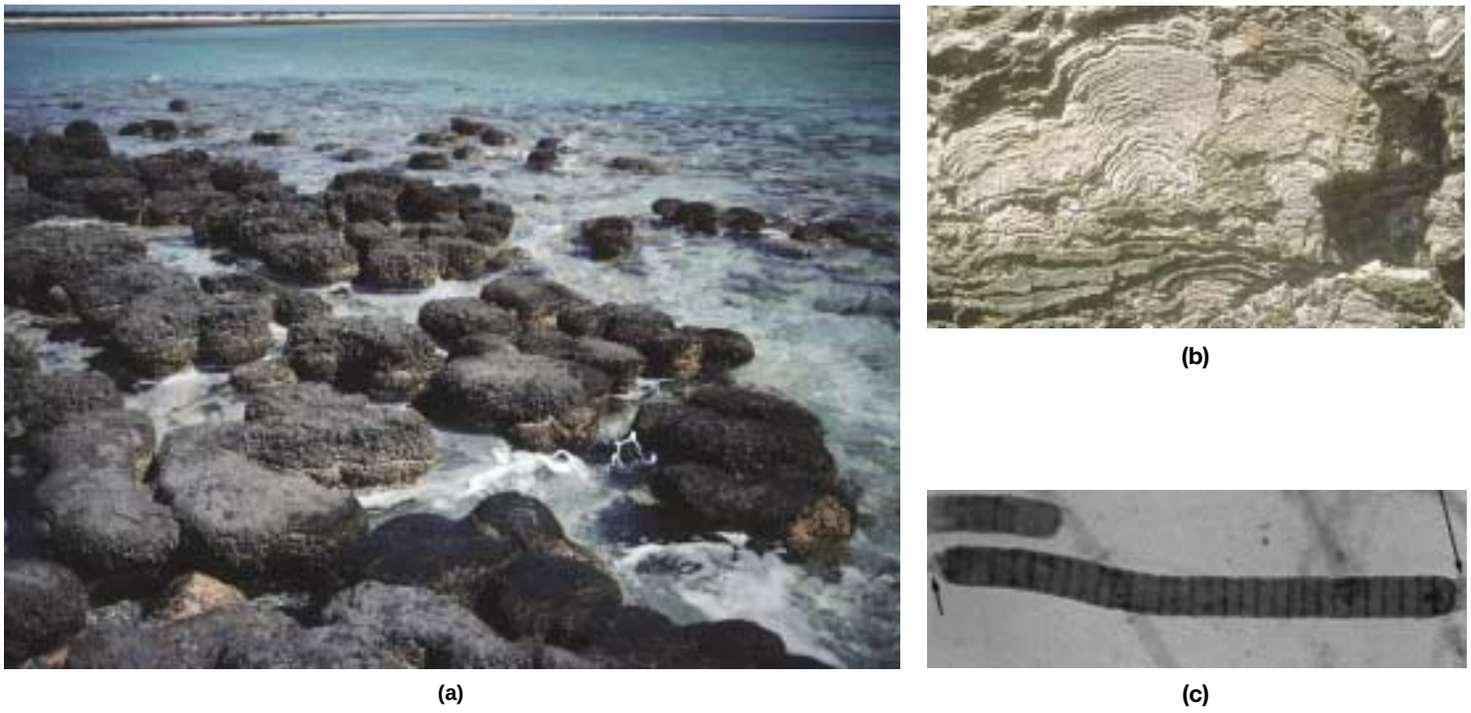
Right now there are more questions than answers. The correct apportioning of multiple chromosomes is not yet understood. Some researchers think it is aided by a mitosis-like process which is not yet well-identified, but relies on hypothetical microfilaments in the cytoplasm. We do know, however, that cells not receiving both kinds of chromosomes continue on for awhile, but eventually die. Maybe there is no "system" for assuring correct distribution, and those who are unlucky just die.

An extreme view of all this gene-swapping and reorganization of genomes is one that regards the entire bacterial universe as a single, huge, superorganism which possesses a network-like structure. A pool of genetic information is accessible to all bacterial cells by means of vertical and horizontal traffic, and is in continuous movement from one part of the superorganism to another. Indeed, for a long time scientists thought that genetic recombination among bacteria was extremely rare, and that mutation was the main driving force of evolution. However, we now must rethink this in view of the far greater frequency of horizontal gene transfer.

Eukaryotic genes enter this pool especially via intracellular endosymbiosis. Bacterial genes are horizontally transferred into host cell chromosomes, which in turn donate some of their genes to the bacterium. Eventually some essential genes of each wind up in the other's genome and both are then incapable of independent existence. Their symbiosis has become compulsory. Pathogenic bacteria sometimes use their pili to insert virulence gene sequences into eukaryotic cells. Parts of these virulence sequences of bacterial origin are not found integrated into eukaryotic chromosomes. So, perhaps we ought not to think of just all bacteria, but of all life, as being one huge superorganism, transferring genetic material among its parts through a network-like structure, rather than being limited to a vertical clonal descent.

Special Methods Needed for Prokaryotes

The taxonomy of most eukaryotes is based on morphology (structural characteristics) of living organisms, genetic features, and on knowledge of their evolutionary re-



(a) (b) (c)

Figure 9.17 Stromatolites. (a) Mats of cyanobacteria growing as stromatolites in shallow seawater off western Australia. These formations are 1,000–2,000 years old. **(b)** A cross-section through fossil stromatolites from Montana, showing horizontal layers of bacterial growth. **(c)** Filamentous cyanobacteria (*Paleolyngbya*) from the Lakhanda Formation in eastern Siberia. These microfossils date from the late Precambrian period and are approximately 950 million years old.

lationships from fossil records. However, morphology and fossil records provide little information about prokaryotes. For one thing, prokaryotes have left few fossil records. As mentioned earlier, stromatolites, fossilized mats of prokaryotes, have been found mainly at sites where the environment millions of years ago allowed the deposition of dense layers of bacteria (Figures 9.17a and b). Stromatolites have provided much of our knowledge of the origin of the Archaea. Unfortunately, most bacteria do not form such mats, so most ancestral prokaryotes have disappeared without a trace.

Chemical clues found in a manganese mine in South Africa suggest that bacteria, algae, or other simple organisms inhabited the soil there 2 billion years ago.

Some rocks containing fossils of individual cells of cyanobacteria have been discovered (Figure 9.17c), but they have failed to reveal much information about the organisms. Moreover, prokaryotes have few structural characteristics, and these characteristics are subject to rapid change when the environment changes. Large organisms tend to require a fairly long period of time to reproduce, but prokaryotes reproduce rapidly. Assuming the same number of mutations per generation, organisms that reproduce most rapidly will accumulate a greater number of mutations over a given period of time. Because of this rapid mutational change rate, it is far more difficult to show the relationship between fossilized forms of prokaryotes and current organisms.

Because morphology and evolution are of little use in classifying prokaryotes, metabolic reactions, genetic relatedness, and other specialized properties have been used instead. Health scientists use these properties to identify infectious prokaryotes in the laboratory, but such identification does not necessarily reflect evolutionary relationships among the organisms. The methods described next are of use in exploring evolutionary relationships. Although the methods are particularly appropriate for eukaryotes, they can be used for prokaryotes as well.

Numerical Taxonomy

Numerical taxonomy is based on the idea that increasing the number of characteristics of organisms that we observe, increases the accuracy with which we can detect similarities among them. If the characteristics are genetically determined, the more characteristics two organisms share, the closer their evolutionary relationship. Although the idea of numerical taxonomy was developed before computers were available, computers allow us to compare large numbers of organisms rapidly and according to many different characteristics. In a simple example of numerical taxonomy, each characteristic is assigned a value of 1 if present and 0 if not present. Characteristics such as reaction to Gram staining, oxygen requirements, presence

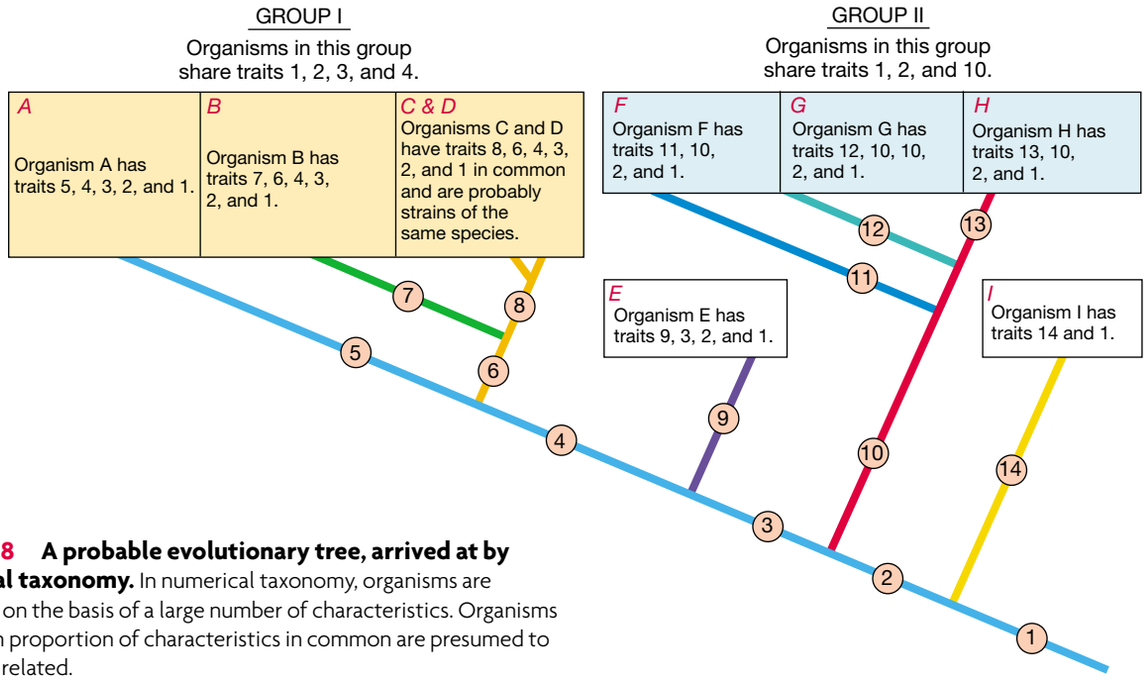


Figure 9.18 A probable evolutionary tree, arrived at by numerical taxonomy. In numerical taxonomy, organisms are compared on the basis of a large number of characteristics. Organisms with a high proportion of characteristics in common are presumed to be closely related.

or absence of a capsule, properties of nucleic acids and proteins, and the presence or absence of particular enzymes and chemical reactions can be evaluated. Organisms are then compared, and patterns of similarities and differences are detected (Figure 9.18) With the use of numerical taxonomy, no single characteristic is used to arbitrarily divide all organisms into groups. If two organisms match on 90% or more of the characteristics studied, they are presumed to belong to the same species. Computerized numerical taxonomy offers great promise for improving our understanding of relationships among all organisms.

Genetic Homology

The discovery of the structure of DNA by James Watson and Francis Crick in 1953 provided new knowledge that was quickly applied by taxonomists, especially those studying taxonomic relationships and the evolution of eukaryotes. These scientists began to study the **genetic homology**, or the similarity of DNA, among organisms. Ideally one could just sequence the entire genome of every organism and compare them all to each other. This, however, is not a practical option at this time. It takes a lot of hard work and time to sequence just one genome—refer back to the website interview with Dr. Karen Nelson. Several faster and easier techniques for estimating genetic homology are available. Similarities in DNA can be studied directly by determining the base composition of the DNA, by sequencing the bases in portions of DNA or RNA, and by using DNA hybridization. Because an organism's proteins are determined by its DNA, similarities in DNA can be studied indirectly by preparing *protein profiles* and by analyzing amino acid sequences in proteins.

BASE COMPOSITION

Organisms can be grouped by comparing the relative percentages of bases present in the DNA of their cells. DNA contains four bases, abbreviated as A (adenine), T (thymine), G (guanine), and C (cytosine) (◀Chapter 2, p. 45). Base pairing occurs only between A and T and between G and C. In making base comparisons, we determine the total amount of G and C in a sample of DNA and express it as a percentage of total DNA. By subtracting this percentage from 100, we get the percentage of total A and T in the sample. For example, if the DNA is 60% G—C, then it is 40% A—T. The base composition of an organism is generally stated in terms of the percentage of guanine plus cytosine and is referred to as the G—C content. Base composition only determines the total amount of each nucleotide base present; it does not give any indication of the sequence of these bases.

Studies of base composition have shown that the G—C content varies from 23 to 75% in bacteria. These studies also have shown that certain species of bacteria, such as *Clostridium tetani* and *Staphylococcus aureus*, have very similar DNA compositions, but that *Pseudomonas aeruginosa* has a very different DNA composition. Thus, *C. tetani* and *S. aureus* are probably more closely related to each other than either is to *P. aeruginosa*. Similar percentages of bases do not in themselves prove that the organisms are

Figure 9.19 **A DNA sequencer.** Automated systems can identify the sequence of nucleotide bases in a piece of DNA.

DNA sequencing is now extremely common. For $20 the Macromolecular Structure Analysis Facility will determine the DNA sequence of your sample.

closely related, because the *sequence* of bases may be quite different. (Human beings and *Bacillus subtilis*, for example, have nearly identical G—C percentages.) We can say, however, that if the percentages in two organisms are quite different, they are not likely to be closely related.

DNA AND RNA SEQUENCING

Automated equipment for identifying the base sequences in DNA or RNA is now available at reasonable cost (Figure 9.19). It is therefore easier than before to search a culture for base sequences known to be unique to certain species. Using PCR techniques and a DNA synthesizer, one can produce a large number of **probes**, single-stranded DNA fragments that have sequences complementary to those being sought (◀Chapter 7, p. 196). A fluorescent dye or a radioactive tag (an indicator molecule) can be attached to the probe. When the probe finds its target DNA, it complementarily binds to it and does not wash off when rinsed. The specimen is then examined for fluorescing dye or for radioactivity. The presence or absence of the unique DNA sequence helps in identification of the specimen.

DNA HYBRIDIZATION

In **DNA hybridization**, the double strands of DNA of each of two organisms are split apart, and the split strands from the two organisms are allowed to combine (Figure 9.20). The strands from different organisms will **anneal** (bond to each other) by base pairing—A with T and G with C. The amount of annealing is directly proportional to the quantity of identical base sequences in the two DNAs. A high degree of homology (similarity) exists when both organisms have long, identical sequences of bases. Close DNA homology indicates that the two organisms are closely related and that they probably evolved from a common ancestor. A small degree of homology indicates that the organisms are not very closely related. Ancestors of such organisms probably diverged from each other thousands of centuries ago and have since evolved along separate lines.

You can use complementary DNA hybridization to detect the presence of DNA sequences from infectious viral agents.

PROTEIN PROFILES AND AMINO ACID SEQUENCES

Every protein molecule consists of a specific sequence of amino acids and has a particular shape with an assortment

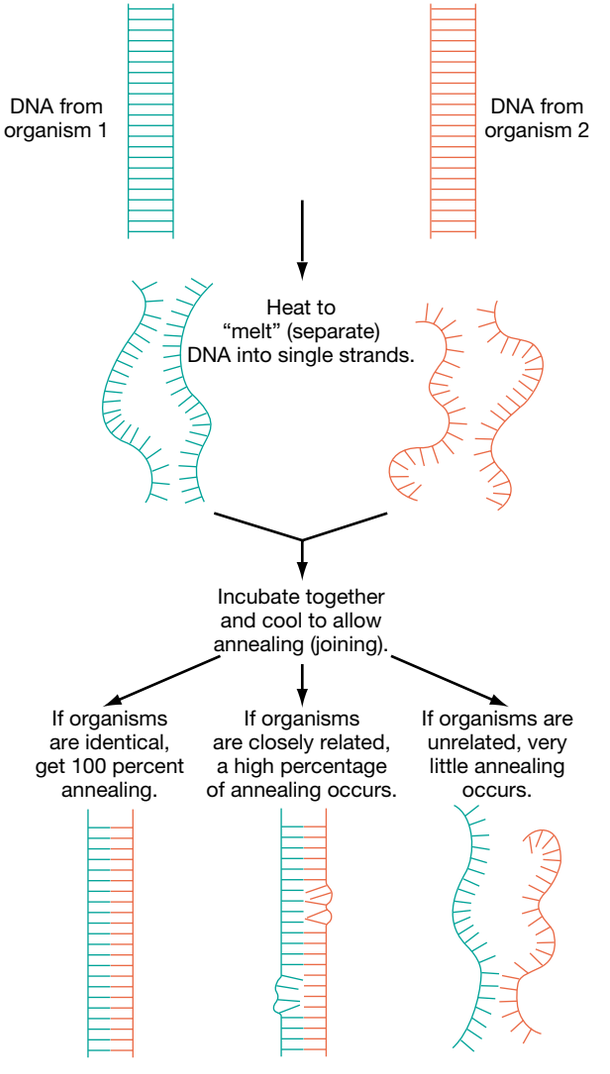


Figure 9.20 **DNA hybridization.** Strands of DNA are separated, and individual strands from two different organisms are allowed to anneal (join by hydrogen bonding at sites where there are many complementary base pairs). The degree of annealing reflects the degree of relatedness between the organisms, based on the assumption that annealing takes place only where genes, or parts of genes, are identical.

of surface charges. Modern laboratory methods allow cells or organisms to be compared according to these properties of their proteins. Although variations in proteins among cells make these techniques difficult to apply to multicellular organisms, they are quite helpful in studying unicellular organisms.

A **protein profile** is a laboratory-prepared pattern of the proteins found in a cell (Figure 9.21a). Because a cell's proteins are the products of its genes, the cells of each species synthesize a unique array of proteins—as distinctive as a fingerprint is for humans. Analysis of the profiles of one or more proteins of different bacterial species provides a reasonable basis for comparisons.

Protein profiles are produced by the **polyacrylamide gel electrophoresis (PAGE)** method, which separates proteins on the basis of molecular size (Figure 9.21b). In this method, samples of protein obtained from lysed cells are dissolved in a detergent and poured into wells (depressions) of a thin slab of polyacrylamide gel. The slab is inserted into a buffer-filled chamber. An electric current is then passed through the gel for a period of time. The current causes the protein molecules to migrate to the opposite end of the gel. Large protein molecules migrate more slowly than do smaller ones. After the smallest proteins have migrated, the current is turned off, and the gel slab is then removed. Next, it is stained so that the various proteins show up as separate stained bands in the gel slab (◀Chapter 18, p. 542).

Each band in the profile from one kind of cell represents a different protein in that cell. Bands at the same location in profiles from different kinds of cells indicate that the same protein is present in the different cells.

Determination of amino acid sequences in proteins also identifies similarities and differences among organisms. Certain proteins, such as cytochromes, which contribute to oxidative metabolism in many organisms, are commonly used to study amino acid sequences. The amino acid sequences in the same kind of protein from several organisms are determined. As with DNA hybridization, the extent of matching sequences of amino acids in the proteins indicates the relatedness of the organisms.

The proteins an organism contains are determined directly by the information in that organism's DNA. Thus, both protein profiles and determinations of amino acid sequences are as significant a measure of the relatedness of organisms as are DNA homologies. All are also related to the evolutionary history of the organisms.

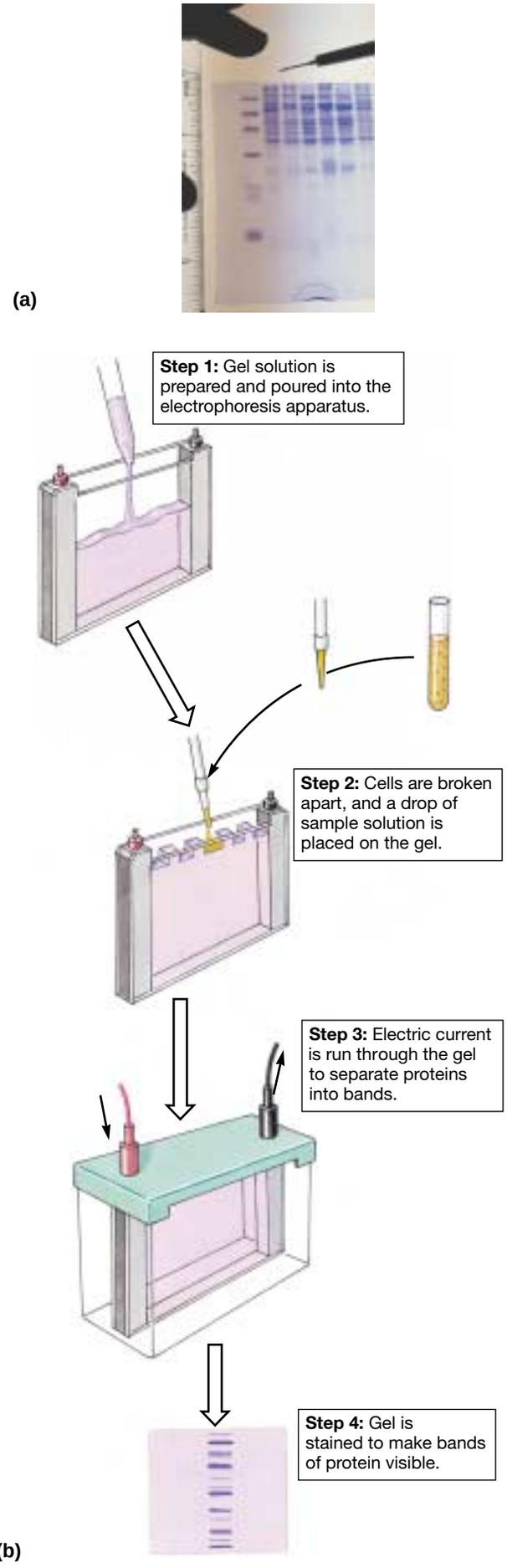


Figure 9.21 Separation of proteins. (a) Protein profiles, which provide a "fingerprint" of the proteins present in particular cells, can be used to compare different organisms to determine their degree of relatedness. **(b)** The PAGE process.

Other Techniques

Other techniques for studying evolutionary relatedness include determining properties of ribosomes, immunological reactions, and phage typing.

PROPERTIES OF RIBOSOMES

Ribosomes serve as sites of protein synthesis in both prokaryotic and eukaryotic cells. RNA in ribosomes can be separated into several types according to the size of the RNA units. A particular RNA unit, the 16S rRNA component, has proven especially useful in studying evolutionary relationships for several reasons. Because rRNA molecules are easily rendered nonfunctional by even slight alterations in their genetic structure, mutations are rarely tolerated, and therefore ribosomes have evolved very slowly. It is the degree of similarity in 16S rRNA sequences between two organisms that indicate their evolutionary relatedness. If the nucleotide sequences of 16S rRNA molecules from two types of organism are very similar, those organisms are likely to be quite close evolutionarily. Although direct sequencing of 16S rRNA is used to show evolutionary relationships between species, newer methods, such as PCR, are beginning to replace it. The PCR technique, which is being used to amplify rRNA genes, requires less cell material and is more rapid and convenient for large studies than is direct rRNA sequencing.

Since ribosomal RNAs are functionally constant in bacteria, we may find drugs that target specific bacterial 16S or rRNA sequences to treat different infections.

IMMUNOLOGICAL REACTIONS

Immunological reactions also are used to identify and study surface structures and the composition of microorganisms, as explained in Chapter 17. As we shall see, one highly specific and sensitive technique involves proteins called *monoclonal antibodies*. Monoclonal antibodies can be created so that they will bind to a specific protein, usually a protein found on a cell surface. If the antibodies bind to the surfaces of more than one kind of organism, the organisms have that protein in common. This technique promises to be particularly useful in identifying specific biochemical properties of microorganisms. In turn, identification of such properties will be extremely useful in determining taxonomic relationships.

PHAGE TYPING

Phage typing involves the use of bacteriophages, viruses that attack bacteria, to determine similarities among different bacteria. A separate agar plate is inoculated for each bacterium being studied. A sterile cotton swab or bent glass rod is used to spread the inoculum over the agar surface. After incubation, a *lawn*, or continuous sheet, of *confluent* bacterial growth will be produced. At the time of swabbing the plate, the underside of the plate is marked with numbered squares so that drops of known phages can be spotted onto specific zones of the plate and later identified. After a suitable incubation period, as the lawn grows up, zones of lysis (*plaques*) appear in the bacterial lawn (Figure 9.22). Because receptor sites for bacteriophages are highly specific, certain strains of a species of bacterium are attacked only by particular types of phages. By observing which phages cause holes in the lawn, researchers can identify the strain. Stains lysed by the same phages are presumed to be more closely related than strains that show different patterns of lysis by phages.

There are 10 times more kinds of phages than kinds of bacteria.

The Significance of Findings

The main significance of methods of determining evolutionary relationships is that these methods can be used to group closely related organisms and to separate them from less closely related ones. When groups of closely related organisms are identified, it is presumed that they probably had a common ancestor and that small differences among them have arisen by *divergent evolution*. **Divergent evolution** occurs as certain subgroups of a species with common ancestors undergo sufficient mutation to be identified as separate species.

Within the eubacteria, an early divergence gave rise to two important subgroups, the Gram-positive bacteria and the Gram-negative ones. Subsequent divergence within each group has given rise to many modern species of bacteria. Among the Gram-negative bacteria, the purple nonsulfur bacteria gave rise to modern bacteria that inhabit animal digestive tracts.

Figure 9.22 Phage typing. Receptor sites for bacteriophages are highly specific; certain strains of a species of bacterium are attacked only by particular types of phages. Clear sites (plaques) are left when phages have killed bacterial cells. On the basis of which phages have attacked a bacterial culture, one can determine which strain of that bacterial species is present.

✓ CHECKLIST

1. What are stromatolites? What can they tell us about the evolution of prokaryotes?
2. Why are genetic homologies more useful than morphology in the study of evolution and classification of microbes?
3. Why is the study of ribosomal RNA especially useful in studying evolutionary relationships among organisms?

BACTERIAL TAXONOMY AND NOMENCLATURE

Criteria for Classifying Bacteria

Most macroscopic organisms can preliminarily be classified according to observable structural characteristics. But it is more difficult to classify microscopic organisms, especially bacteria, because many of them have similar structures. Separating them according to cell shape, size, and arrangement does not produce a very useful classification system. Nor does the presence of specific structures such as flagella, endospores, or capsules allow identification of particular species. Therefore, other criteria must be used. Staining reactions, especially the Gram stain, were among the first properties other than morphology to be used to classify bacteria. Other properties now in use include features related to growth, nutritional requirements, physiology, biochemistry, genetics, and molecular analysis. These features include properties of DNA and proteins. Important criteria used in classifying bacteria are summarized in Table 9.5, and biochemical tests used in classifying and identifying them are described in Table 9.6.

By using various classification criteria, we can identify an organism as belonging to a particular genus and species. For bacteria, a species is regarded as a collection of strains that share many common features and differ significantly from other strains. A bacterial *strain* consists of descendants of a single isolation in pure culture. Bacteriologists designate one strain of a species as the **type strain**. Usually this is the first strain described. It is the name-bearer of the species and is preserved in one or more type culture collections. The American Type Culture Collection (ATCC), a nonprofit scientific organization established in 1925, collects, preserves, and distributes authenticated type cultures of microorganisms. Many important research studies dealing with classification, identification, and the industrial uses of microorganisms would be seriously hampered without the services of the ATCC.

For many strains of bacteria, scientists are able to determine that they are members of a particular species. For other strains, however, difficult judgments must be made to decide whether the strain belongs to an existing species or differs sufficiently to be defined as a separate species. In recent years, similarities of DNA and proteins among or-

TABLE 9.5

Criteria for Classifying Bacteria

Criteria	Examples	Uses
Morphology	Size and shape of cells; arrangements in pairs, clusters, or filaments; presence of flagella, pili, endospores, capsules	Primary distinction of genera and sometimes species
Staining	Gram-positive, Gram-negative, acid-fast	Separates eubacteria into divisions
Growth	Characteristics in liquid and solid cultures, colony morphology, development of pigment	Distinguish species and genera
Nutrition	Autotrophic, heterotrophic, fermentative with different products; energy sources, carbon sources, nitrogen sources, needs for special nutrients	Distinguish species, genera, and higher groups
Physiology	Temperature (optimum and range); pH (optimum and range), oxygen requirements, salt requirements, osmotic tolerance, antibiotic sensitivities and resistances	Distinguish species, genera, and higher groups
Biochemistry	Nature of cellular components such as cell wall, RNA molecules, ribosomes, storage inclusions, pigments, antigens; biochemical tests	Distinguish species, genera, and higher groups
Genetics	Percentage of DNA bases (G + C ratio); DNA hybridization	Determine relatedness within genera and families
Serology	Slide agglutination, fluorescent-labeled antibodies	Distinguish strains and some species
Phage typing	Susceptibility to a group of bacteriophages	Identification and distinguishing of strains
Sequence of bases in rRNA	rRNA sequencing	Determine relatedness among all living things
Protein profiles	Separate proteins by two-dimensional PAGE (electrophoresis)	Distinguish strains

TABLE 9.6

Specific Biochemical Tests Sometimes Used in Identifying and Classifying Bacteria

Biochemical Test	Nature of Test
Sugar fermentation	Organism is inoculated into a medium containing a specific sugar; growth and end products of fermentation, including gases, are noted. Anaerobic fermentations can be detected by inoculating organisms via a "stab" culture into solid medium.
Gelatin liquefaction	Organism is inoculated (stabbed) into a solid medium containing gelatin; liquefaction at room temperature or inability to resolidify at refrigerator temperature indicates the presence of proteolytic (protein-digesting) enzymes.
Starch hydrolysis	Organism is inoculated onto an agar medium containing starch; after the plate is flooded with Gram's iodine, clear areas around colonies indicate the presence of starch-digesting enzymes.
Litmus milk	Organism is inoculated into litmus milk medium (10% powdered skim milk plus litmus indicator); characteristic changes such as alteration of pH to acid or alkaline, denaturation of the protein casein (curdling), and gas production can be used to help identify specific organisms.
Catalase	Hydrogen peroxide (H_2O_2) is poured over heavy growth of an organism on an agar slant; release of O_2 gas bubbles indicates the presence of catalase, which oxidizes H_2O_2 to H_2O and O_2.
Oxidase	Two or three drops (or a disk) of an oxidase test reagent is added to an organism growing on an agar plate; a color change of the test reagent to blue, purple, or black indicates the presence of cytochrome oxidase.
Citrate utilization	Organism is inoculated into citrate agar medium in which citrate is the sole carbon source; an indicator in the medium changes color if citrate is metabolized; use of citrate indicates the presence of the permease complex that transports citrate into the cell.
Hydrogen sulfide	Organism is inoculated into peptone iron medium; formation of black iron sulfide indicates the organism produces hydrogen sulfide (H_2S).
Indole production	Organism is inoculated into a medium containing the amino acid tryptophan; production of indole, a nitrogenous breakdown product of tryptophan, indicates the presence of a set of enzymes that convert tryptophan to indole.
Nitrate reduction	Organism is inoculated into a medium containing nitrate (NO_3^-); presence of nitrite (NO_2^-) indicates that the organism has the enzyme nitrate reductase; absence of intrite indicates either absence of nitrate reductase or presence of nitrite reductase (which reduces nitrite to N_2 or NH_3).
Methyl red	Organism is cultured in MR-VP broth; a methyl red indicator is added; presence of acid causes an indicator color change (red).
Voges-Proskauer	Organism is cultured in MR-VP broth; alpha naphthol and KOH-creatine are added; presence of the enzyme cytochrome oxidase causes color change in an indicator (rose color).
Phenylalanine deaminase	Organism is inoculated into a medium containing phenylalanine and ferric ions; formation of phenylpyruvate and its reaction with ferric ions produces a color change that demonstrates the presence of the enzyme phenylalanine deaminase.
Urease	Organism is inoculated into a medium containing urea; production of ammonia, usually detected by an indicator for alkaline pH, indicates the presence of the enzyme urease.
Specific nutrient	Organism is inoculated into a medium containing a specific nutrient, such as a particular amino acid (e.g., cysteine) or vitamin (e.g., niacin); growth of an organism that fails to grow in media lacking the specific nutrient can be used to identify some auxotrophs.

ganisms have proved a reliable means of assigning a strain to an existing species or establishing the basis for a new species.

Curiously, assigning bacterial genera to higher taxonomic levels—families, orders, classes, and divisions (or phyla)—can be even more difficult than organizing species and strains *within* genera. Many macroscopic organisms are classified by establishing their evolutionary relationships to other organisms from fossil records. Efforts are being made to classify bacteria by evolutionary relationships, too, but these efforts are hampered by the incompleteness of the fossil record and by the limited information gleaned from what fossils have been found. Even a complete fossil record might supply only morphological information and would thus be inadequate for determining evolutionary relationships.

The History and Significance of *Bergey's Manual*

The accepted reference on the identification of bacteria is commonly referred to as *Bergey's Manual*. The first edition of *Bergey's Manual of Determinative Bacteriology*

Figure 9.23 David H. Bergey, the originator of *Bergey's Manuals*. The first *Bergey's Manual* was published in 1923. In 1936 he set up an educational trust to which all rights and royalties from the *Manuals* would be transferred for preparing, editing, and publishing future editions, as well as providing funds for research to clarify problems arising in the process. This nonprofit trust ensures that *Bergey's Manual* will be a self-perpetuating publication.

was published in 1923 by the American Society for Microbiology; David H. Bergey (Figure 9.23) was chairperson of its editorial board. Since then, eight editions, an abridged version, and several supplements have been published. The *determinative information* (information used to identify bacteria) was collected into a single volume, the ninth edition of *Bergey's Manual of Determinative Bacteriology*, which was published in 1994. *Bergey's Manual* has become an internationally recognized reference for bacterial taxonomy. It has also served as a reliable standby for medical workers interested in identifying the causative agents of infections.

A four-volume first edition of *Bergey's Manual of Systematic Bacteriology* was published between 1984 and 1989, having a much broader scope. It provided descriptions and photographs of species, tests to distinguish among genera and species, DNA relatedness among organisms, and various numerical taxonomy studies (Figure 9.24).

However, it is important to remember that, in their current state, both *Bergey's Manuals* do *not* present an accurate picture of evolutionary relationships among bacteria. Rather, they are practical groupings of bacteria that make it easy to identify them. We did not yet have enough information to draw a complete evolutionary tree for bacteria.

The five-volume second edition of *Bergey's Manual of Systematic Bacteriology* (Appendix B) represents a major departure from the first edition, as well as from the eighth and ninth editions of *Bergey's Manual of Determinative Bacteriology*. It is based on a phylogenetic (evolutionary) framework, rather than on a nonevolutionary grouping by phenotype. Sequencing of 16S rDNA has provided guidance in this, but it is still very much a "work in progress." Indeed, its editors expect discovery of well over 100 new genera in the 5 or 6 years between publication of the first and last volumes of the *Manual*. They also acknowledge that some taxa are "problematic," containing misidentified species, and that some phylogenetic trees are ambiguous. Therefore at the publication of each subsequent volume, they will publish a new taxonomic outline based on the then-current understanding of relationships. The web site of the Bergey's Manual Trust will be regularly updated to show these changes. The web address is www.cme.msu.edu/bergeys. Since publication of the first edition in 1984, over 2,300 new species and 400 new genera, plus 850 other name changes have occurred. The future pace will be even more rapid. Volume One was published in May 2001; Volume Two in February 2004. Appendix B gives the taxonomic outline as of April 20, 2001. The entire series of volumes will cover:

Volume 1: *The Archaea and the Deeply Branching and Phototrophic Bacteria*, covers Phyla A1, A2, and B1 through B12.

Volume 2: *The Proteobacteria* (Phylum B12)

Volume 3: *The Low G+C Gram Positives* (Phylum B13)

Volume 4: *The High G+C Gram Positives* (Phylum B14)

Volume 5: *The Planctomycetes, Spirochaetes, Fibrobacteres, Bacteroidetes and Fusobacteria* (Phyla B15 through B23).

The new taxonomic outline is so different that Bergey's web site actually offers an index to assist you in finding the places to which old familiar genera have been moved. Another change is that the term "Kingdom" is no longer used. When all five second edition volumes have been published, the tenth edition of *Bergey's Manual of Determinative Bacteriology*, using this information will be published. Meanwhile, in the coming years, we shall all have to remain fluid, and "go with the flow" of where the new taxonomy leads us. And keep your first edition handy for a few more years!

CLOSE-UP

Happy Hunting

Most people have heard about Dolly, the cloned sheep, or Mr. Jefferson, the cloned calf. With successful genetic discoveries and experiments like these going on, you probably assumed that most of the organisms inhabiting the Earth were well known. But that's not true. Biology is still discovering basic information about the most abundant, widely distributed, and biochemically versatile organisms on the planet—the prokaryotes. Though prokaryotes have been thriving on Earth for over 3.5 billion years; play key roles in the chemical transformations of carbon, nitrogen, and sulfur in our biosphere; and live everywhere, even in bizarre and extreme habitats, prokaryotes are probably the least understood organisms on Earth. One recent study of a single habitat, for example, revealed a large variety of new bacterial groups, nearly doubling the number of bacterial phyla! Microbiologists need not fear—there is still a vast and largely unexplored microbial world to discover.

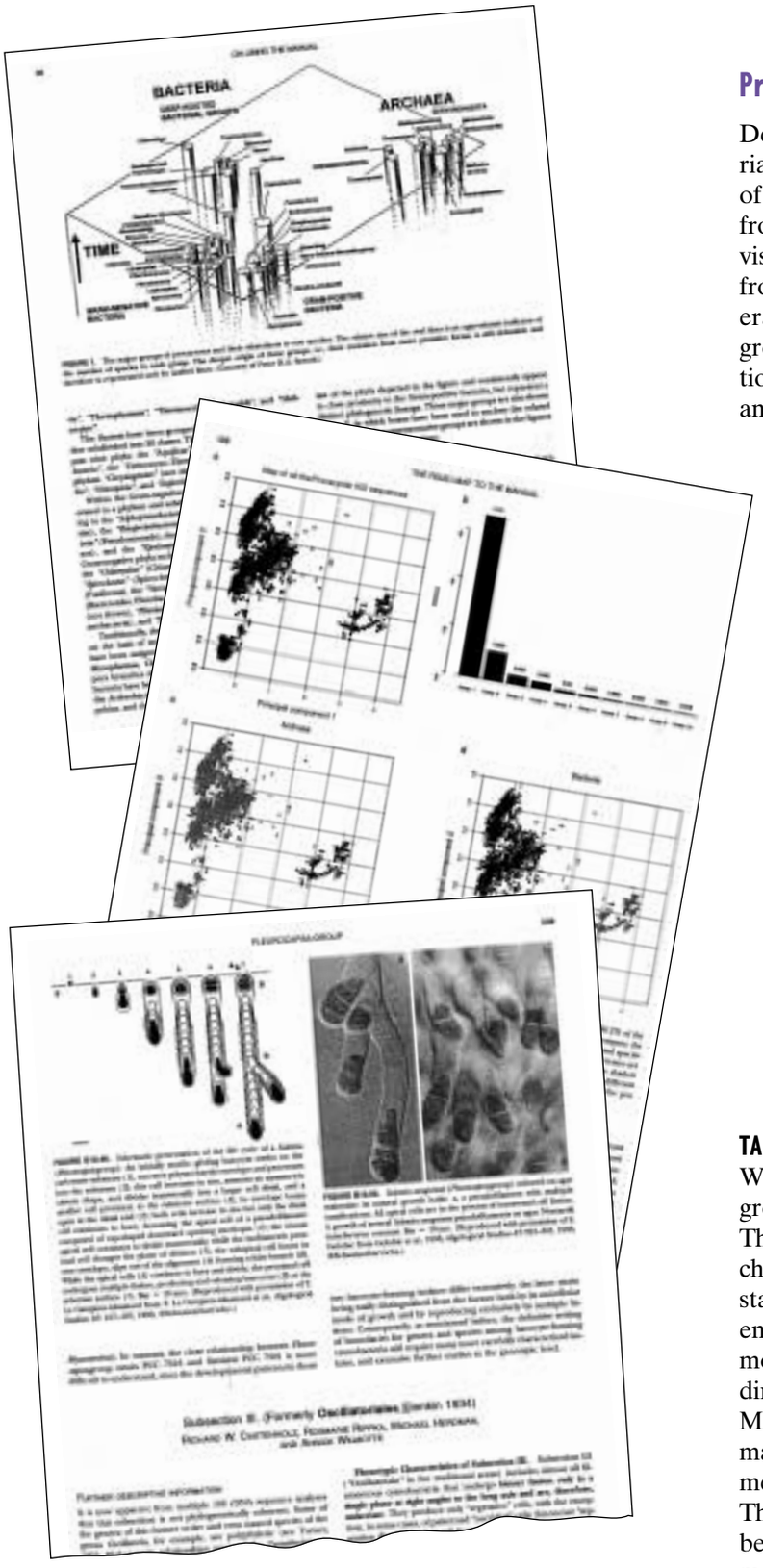


Figure 9.24 **Samples from *Bergey's Manuals*.** Shown here are three representative pages reproduced from the five-volume set of *Bergey's Manual of Systematic Bacteriology*. (top) Major groups of prokaryotes and their relatedness to one another. (middle) Map of evolutionary distances between various Archaea and Bacteria based on DNA sequences. (bottom) Life cycle of *Solentia*, an unusual bacterium that forms pseudofilaments composed of downward-opening envelopes.

Problems Associated with Bacterial Taxonomy

Despite the tremendous effort spent in classifying bacteria, the plight of bacteriologists looking at the taxonomy of bacteria might be described as follows: Those looking from the top level down can propose at least plausible divisions of the prokaryotes. Those bacteriologists looking from the bottom up can establish strains, species, and genera and can sometimes assign bacteria to higher-level groups. But too little is known about evolutionary relationships to establish clearly defined taxonomic classes and orders for many bacteria.

TAXONOMY FROM THE TOP DOWN

Bergey's Manual of Systematic Bacteriology first edition divided the kingdom Prokaryotae into the following four divisions on the basis of cell wall properties:

Division I	Gracilicutes (gras″ih-lik-yoo′teez)	Prokaryotes with thin cell walls, which are typical of Gram-negative organisms
Division II	Firmicutes (fer-mik′ yoo-teez)	Prokaryotes with thick cell walls, indicating a Gram-positive type of cell wall
Division III	Tenericutes (ten″er-ik′ yoo-teez)	Prokaryotes of a pliable, soft nature, and lacking cell walls
Division IV	Mendosicutes (men-doh-sik′yoo-teez)	Prokaryotes having cell walls that lack peptidoglycan

TAXONOMY FROM THE BOTTOM UP

Within each division, bacteria were classified into 33 groups, called "sections," in *Bergey's Manual* first edition. The sections are based on relatively easily observable characteristics such as shape, staining reactions, the presence or absence of a cell wall, motility, reproduction by budding, and mode of metabolism. Most sections were established many years ago as a practical means of classifying bacteria. The criteria were established before modern techniques for molecular analysis (especially of DNA and RNA) and biochemical studies were available, and before any fossil bacteria had been found.

New bacteria are constantly being discovered. Scientists estimate the number of as-yet-unknown species is in the millions.

As more and more bacteria have been subjected to modern analysis, certain discrepancies in assignments to sections have become apparent. For example, the cell walls of some genera have unusual properties that prevent their being classified as either Gram-positive or

Gram-negative. The organisms assigned to a particular section of *Bergey's Manual* first edition are not necessarily closely related. The second edition strives to correct this.

THE MUDDLE IN THE MIDDLE

The difficulties of classifying bacteria are greatly magnified as one proceeds with total genome sequencing and discovery of more and more examples of lateral gene transfer. Although families, orders, and classes can be clearly defined for a few bacteria, too little information is available to do so for many. Until discrepancies in section assignments can be resolved and taxonomic levels between genera and phyla can be more precisely determined and published, it is practical to continue to use first edition section designations. The medically important sections are summarized in Table 9.7.

TABLE 9.7

Characteristics and Medically Important Members of Selected Sections of Bacteria Defined in *Bergey's Manual of Systematic Bacteriology*

Section (Number)	Medically Important Members	Diseases or Products
Spirochetes (1): Gram-negative, helical, move by axial filaments	*Treponema*	Syphilis
	Borrelia	Relapsing fever; Lyme disease
	Leptospira	Leptospirosis
Aerobic, Motile, Helical Gram-Negative Bacteria (2): move by flagella, helical or comma-shaped	*Campylobacter*	Urogenital and digestive tract infections
	Helicobacter	Peptic ulcers
Gram-Negative Aerobic Rods and Cocci (4): Some contain pigments or oxidase, some have fastidious nutritional requirements, some are obligate parasites	*Pseudomonas*	Urinary tract infections, burns, and wounds
	Legionella	Pneumonia and other respiratory infections
	Neisseria	Gonorrhea, meningitis, and nasopharyngeal infections
	Moraxella	Conjunctivitis
	Brucella	Brucellosis
	Bordetella	Whooping cough
	Francisella	Tularemia
Facultatively Anaerobic Gram-Negative Rods (5): Some have peritrichous flagella, many can be distinguished by their characteristic fermentation reactions	*Escherichia*	Opportunistic infections of colon and other sites
	Shigella	Bacillary dysentery
	Salmonella	Typhoid fever, enteritis, and food poisoning
	Klebsiella	Respiratory and urinary tract infections
	Enterobacter	Opportunistic infections
	Serratia	Opportunistic infections
	Proteus	Urinary tract infections (especially nosocomial)
	Providencia	Wound and burn infections, urinary tract infections
	Morganella	Summer diarrhea, opportunistic infections
	Yersinia	Plague, mesenteric lymphadenitis, and septicemia
	Vibrio	Cholera, acute gastroenteritis
	Pasteurella	Cat- and dog-bite wounds
	Haemophilus	Respiratory infections, meningitis, conjunctivitis, and chancroid
	Calymmatobacterium	Granuloma inguinale
	Gardnerella	Vaginitis
	Eikenella	Wound infections
	Streptobacillus	Rat-bite fever
Anaerobic Gram-Negative Rods (6): straight, curved, or helical, motile	*Bacteroides, Fusobacterium*	Oral, digestive, respiratory, and urogenital infections, wounds, and abscesses
Anaerobic Gram-Negative Cocci (8): nonmotile	*Veillonella*	Oral microbiota and abscesses
Rickettsiae and Chlamydiae (9): intracellular parasites; chlamydiae form reticulate and elementary bodies	*Rickettsia*	Typhus, Rocky Mountain spotted fever, rickettsialpox
	Rochalimaea	Trench fever
	Coxiella	Q fever
	Bartonella	Oroya fever
	Chlamydia	Trachoma, inclusion conjunctivitis, nongonococcal urethritis, lymphogranuloma venereum, parrot fever

(Continued)

TABLE 9.7

(Continued)

Section (Number)	Medically Important Members	Diseases or Products
Mycoplasmas (10): lack cell walls, extremely small	*Mycoplasma*	Atypical pneumonia, urogenital infections
	Ureaplasma	Opportunistic urogenital infections
Gram-Positive Cocci (12): aerobic to strictly anaerobic; non-spore-forming; typically pyogenic (pus forming)	*Staphylococcus*	Skin abscesses, opportunistic infections
	Streptococcus	Strep throat and other respiratory infections, skin and other abscesses, puerperal fever, opportunistic infections
	Peptococcus	Postpartum septicemia, visceral lesions
	Peptostreptococcus	Puerperal fever and various pyogenic infections
Endospore-Forming, Gram-Positive Rods and Cocci (13): aerobic to strictly anaerobic; some motile and some nonmotile	*Bacillus*	Anthrax, food poisoning; source of the antibiotic bacitracin
	Clostridium	Tetanus, botulism, gas gangrene, and bacteremia
Regular Nonsporing Gram-Positive Rods (14): facultatively or strictly anaerobic; nonmotile	*Lactobacillus*	Microflora of the digestive tract and vagina
	Listeria	Listeriosis
	Erysipelothrix	Erysipeloid
Irregular Nonsporing Gram-Positive Rods (15): club-shaped, pleomorphic, filamentous; aerobic to anaerobic	*Corynebacterium*	Diphtheria and skin opportunists
	Propionibacterium	Wound infections and diseases
	Eubacterium	Oral and other infections
	Actinomyces	Actinomycoses
Mycobacteria (16): Gram-positive, acid-fast	*Mycobacterium*	Tuberculosis, leprosy, and chronic infections
Nocardioforms (17): Gram-positive, filamentous, some acid-fast	*Nocardia*	Nocardiosis, mycetoma, and abscesses
Actinomycetes That Divide in More Than One Plane (27): Gram-positive, filamentous	*Dermatophilus*	Skin lesions
Streptomycetes and Their Allies (29): Gram-positive, filamentous	*Streptomyces*	Produce over 500 different antibiotics

Bacterial Nomenclature

Despite all the taxonomic problems, there is an established nomenclature for bacteria. *Bacterial nomenclature* refers to the naming of species according to internationally agreed-upon rules. Both taxonomy and nomenclature are subject to change as new information is obtained. Organisms are sometimes moved from one category to another, and their official names are sometimes changed. For example, the bacterium that causes tularemia, a fever acquired by handling infected rabbits, was for many years called *Pasturella tularensis*. Its genus name was changed to *Francisella* after DNA hybridization studies revealed that hybridization between its DNA and that of *Pasteurella* species did not occur. It does, however, have a 78% match with the DNA of *Francisella novicida*. When considering specific orders and families, we must remember that such names have consistent endings: orders always end in *-ales* and families in *-aceae*.

Bacteria by Section of *Bergey's Manual*, First Edition

Of the 33 sections of prokaryotes described in *Bergey's Manual of Systematic Bacteriology*, first edition, some contain only a few organisms, whereas others contain hundreds. Certain sections contain organisms of medical significance, whereas others are of interest mainly to ecologists, taxonomists, or researchers.

Some sections, such as Section 9, **Rickettsiae** and **Chlamydiae**, contain rather unusual organisms. These two groups are obligate intracellular parasites; that is, they can only grow inside living cells. Chlamydiae have an interesting and complex life cycle (Figure 9.25) rather than dividing by binary fission, as do Rickettsiae and most other bacteria. **Mycoplasmas** (Section 10) lack cell walls and form colonies that look like eggs fried sunny-side up. They have sterols in their cell membranes that give them great flexibility of shape (pleomorphism; ◀Chapter 4, p. 78). Also in Section 10 are the **Ureaplasmas**, also with unusual cell walls and/or cell membranes. Table 9.8 compares these groups with more typical bacteria and viruses.

Bacterial Taxonomy and You

As a beginning student, you will doubtless find it difficult to remember many characteristics of specific microorganisms that we cover in this course. A four-volume set of *Bergey's Manual, First Edition* weighs about 21 pounds

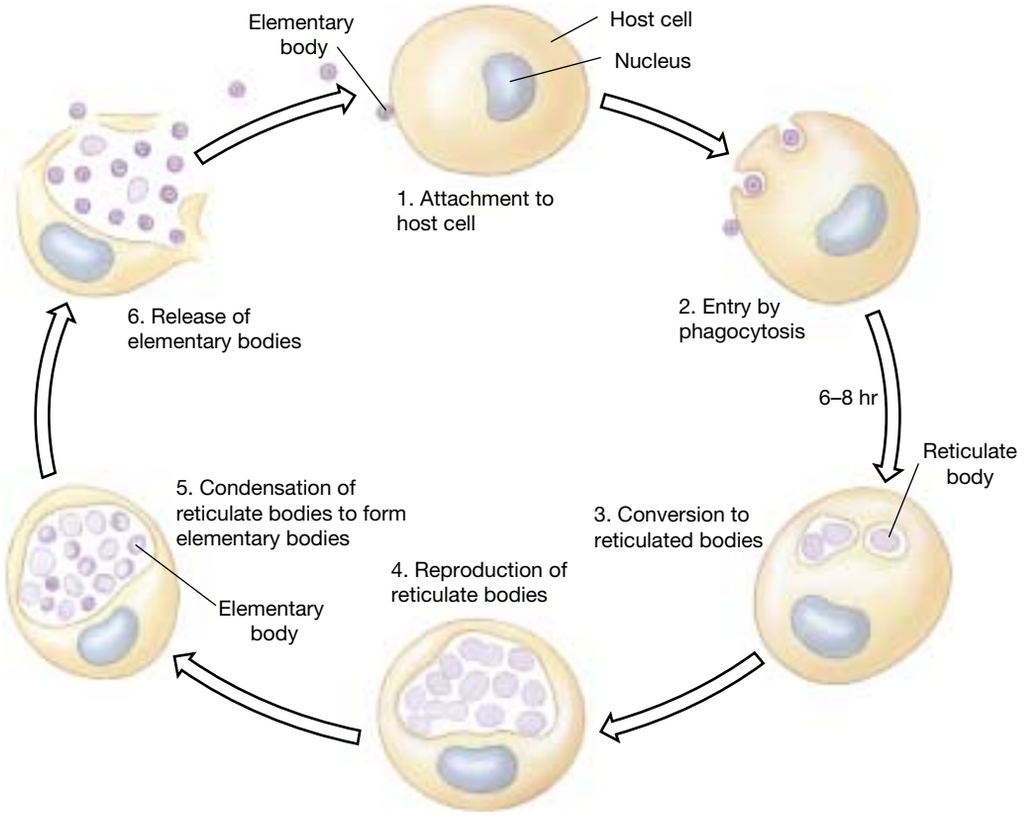


Figure 9.25 The life cycle of a chlamydia. (Step 1) Small, dark elementary bodies (the only infectious stage of the chlamydial life cycle) attach to a host cell and **(2)** enter by phagocytosis. **(3)** The elementary bodies, enclosed within membrane-enclosed vacuoles, lose their thick walls and enlarge to become reticulate bodies. **(4)** Reticulate bodies reproduce by binary fission, rapidly filling the cell. **(5)** They condense to form infectious elementary bodies, which **(6)** are then released by lysis and are free to attach to a new host cell.

and costs about $400—not something you could carry to class and back. And the second edition is mainly unpublished. However, you can use the endpapers, located inside the front and back covers of this textbook. If you wish to find out whether a given organism is Gram-positive or Gram-negative, its shape, the disease(s) it causes, and so on, look it up by name in the back endpapers. Microorganisms are grouped as bacteria, viruses, fungi, and parasites (protozoa and helminths). Or if you are discussing a disease but can't remember which organism(s) cause it, look in the front endpapers under the name of the disease (again grouped as bacterial, viral, fungal, and parasitic diseases), and you will find the organism's name and some of its characteristics. Page numbers are given to direct you to further information. Thumbing through the book or index can be frustrating when you need some little piece of information. But flipping to the cover of your book is easy, and we encourage you to do so often, until the information gradually becomes more and more familiar.

✓CHECKLIST

1. What is a type strain and a type culture collection? Why is such a collection essential to researchers?
2. Why was much of the first edition of *Bergey's Manual* not phylogenetically arranged?
3. What types of information are contained in *Bergey's Manual of Determinative Bacteriology*, compared to *Bergey's Manual of Systematic Bacteriology*?

TABLE 9.8

Characteristics of Typical Bacteria, Rickettsiae, Chlamydiae, Mycoplasmas, Ureaplasmas, and Viruses

Characteristic	Typical Bacteria	Rickettsiae	Chlamydiae	Mycoplasmas	Ureaplasmas	Viruses
Cell wall	Yes	Yes	Yes	No	Sometimes	No
Grow only in cells	No	Yes	Yes	No	No	Yes
Require sterols	No	No	No	Sometimes	Yes	No
Contain DNA and RNA	Yes	Yes	Yes	Yes	Yes	No
Have metabolic system	Yes	Yes	Yes	Yes	Yes	No

CLOSE-UP

The Discovery of New Organisms

Are there any new worlds to discover, or new creatures in them? Yes! In recent years, scientists have discovered living organisms in such diverse environments as submarine hot vents, inside volcanoes, and in deep oil wells. In 1990 a joint U.S. and Soviet team discovered hot vents in fresh water for the first time, complete with an associated community of archaeobacteria, worms, sponges, and other organisms.

The vents lie more than 400 meters deep in a most unusual Russian lake, Lake Baikal, which is the deepest lake in the world and holds the greatest quantity of fresh water in the world. Located in central Asia, in Siberia, it lies in a pocket between two continental plates. Asia was formed as a solid mass when several plates collided one after another and remained together. The area of Lake Baikal is being pulled apart, forming a rift valley and eventually a new ocean. This region is comparable to the seafloor spreading centers (ridges) of the Pacific Ocean, where other vent communities have been found. In both locations, hot materials from deep inside the Earth are emerging. Lake Baikal is a unique treasure for studying the evolution of life and microbial forms. Most lakes are only thousands of years old, but Lake Baikal may be 25 million years old. Microbes similar to the early evolving stages of life may still exist in its depths.

What lives inside a volcano? Studies following the 1980 volcanic eruptions of Mount St. Helens have raised some interesting questions. Archaeobacteria, which had previously been known from the deep-sea volcanic vents (black smokers) located 2,200 meters below the sea surface, have been found living on and in Mount St. Helens at temperatures of 100°C. Where did they come from? Some scientists think that they may have been present deep inside the volcano. For that matter, where do the archaeobacteria in the submarine vents come from? Do they point out a linkage between terrestrial and submarine volcanic activity? We tend to think of life as being present only on the *surface* of the Earth, but perhaps there is a whole different range of life that we know nothing about, deep *within* the crust of the Earth. Daily, more and more evidence accumulates in favor of the idea of "continuous crustal culture."

Cores taken from the deepest oil wells being bored into the Earth reveal archaeobacteria at sites not connected with volcanic activities. Ancient bacteria from the early stages of our planet's development may still be colonizing the hot anaerobic interior of the Earth—places with conditions that resemble those formerly on the Earth's surface.

Mount St. Helens, shown here erupting in July 1980, is home to archaeobacteria.

Various ecological problems have sent out scientists from universities, government, and industry to hunt for new microbes with properties that make the organisms useful in cleaning up the environment. Scientists from the Woods Hole Oceanographic Institution in Massachusetts took their search to a depth of more than 1,800 meters in the Gulf of California, where they have discovered anaerobic bacteria that can degrade naphthalene and possibly other hydrocarbons that might be found in oil spills. Sites in need of bioremediation often lack oxygen, making it impossible to utilize aerobic organisms for cleanup—hence the hunt in deep anaerobic environments. General Electric has also found an anaerobic bacterium that it plans to use to destroy polychlorinated biphenyls (PCBs), industrial by-product chemicals that accumulate in animal tissue and cause damage, including cancer and birth defects.

A new bacterium, so far referred to as GS-15, discovered in the Potomac River by U.S. Geological Survey (USGS) scientists, ordinarily changes iron from one form to another. It seems, however, that these bacteria can just as easily feed on uranium, getting twice as much energy in the process and transforming the uranium into an insoluble precipitate. The USGS team plans to use GS-15 to remove uranium from contaminated well and irrigation water found in much of the western United States and at uranium mining, processing, and nuclear waste sites.

There are many new microbes yet to be discovered. In addition to the naturally occurring species, new ones will be designed by scientists using genetic engineering techniques—or possibly even discovered on another planet! All these species will need to be classified and named. Clearly, *Bergey's Manual* will never be "finished."

RETRACING OUR STEPS

TAXONOMY: THE SCIENCE OF CLASSIFICATION

- Organisms are named according to their characteristics, where they are found, who discovered them, or what disease they cause. **Taxonomy** is the science of classification, and each category is a **taxon**.

Linnaeus, the Father of Taxonomy

- Linnaeus developed the system of **binomial nomenclature**, a two-name identification system for each living organism.
- The **genus** and **specific epithet** of each organism identify the **species** to which it belongs.

- Linnaeus also established the hierarchy of taxonomy and classified organisms into two kingdoms, Plantae and Animalia.

Using a Taxonomic Key

- A **dichotomous key** consists of a series of paired statements presented as either-or choices that describe characteristics of organisms. By selecting appropriate statements to progress through the key, one can classify organisms and, if the key is sufficiently detailed, identify them by genus and species.

Problems in Taxonomy

- Ideally, organisms should be classified by their **phylogenetic**, or evolutionary, relationships.
- Problems in taxonomy include the rapid pace of evolutionary change in microorganisms and the difficulty in deciding what constitutes a kingdom and what constitutes a species.

Developments Since Linnaeus's Time

- Since Linnaeus's time, several taxonomists have proposed three- and four-kingdom systems on the basis of various fundamental characteristics of living things. Whittaker proposed a five-kingdom system in 1969.
- Since 1925, *Bergey's Manual of Determinative Biology* has served as an important tool in identifying bacteria.

THE FIVE-KINGDOM CLASSIFICATION SYSTEM

- The kingdoms of the **five-kingdom system** are **Monera** (**Prokaryotae**), **Protista**, **Fungi**, **Plantae**, and **Animalia**. The characteristics of members of each kingdom are summarized in Table 9.2.

Kingdom Monera

- All monerans are unicellular **prokaryotes**: They generally lack organelles, have no true nuclei, and their DNA has little or no protein associated with it.
- The **cyanobacteria** are photosynthetic monerans of great ecological importance.

Kingdom Protista

- The protists are a diverse group of mostly unicellular **eukaryotes**.

Kingdom Fungi

- The fungi include some unicellular and many multicellular organisms that obtain nutrients solely by absorption.

Kingdom Plantae

- Most plants live on land and contain chlorophyll in organelles called chloroplasts.

Kingdom Animalia

- All animals are derived from zygotes; most are macroscopic.

THE THREE-DOMAIN CLASSIFICATION SYSTEM

- The three **Domains** are higher than the category of Kingdom. They include: **Bacteria**, **Archaea**, and **Eukarya**. Their characteristics are summarized in Table 9.3.
- The concept of a Universal Common Ancestor with a linear *tree of life* has now been replaced by a **shrub of life** with many roots, due to lateral gene transfer.

Domain Bacteria

- All Bacteria are unicellular prokaryotes and include the eubacteria ("true bacteria").

Domain Archaea

- All Archaea are unicellular prokaryotes, having a cell wall made of materials other than peptidoglycan.

Domain Eukarya

- All are eukaryotic cells, having a true nucleus.

CLASSIFICATION OF VIRUSES

- **Viruses**, acellular infectious agents that share only a few characteristics with living organisms, are not included in any of the five kingdoms. Viruses are classified by their nucleic acids, chemical composition, and their morphology.

THE SEARCH FOR EVOLUTIONARY RELATIONSHIPS

Special Methods Needed for Prokaryotes

- Special methods are needed for determining evolutionary relationships among prokaryotes because they have few morphological characteristics and have left only a sparse fossil record.
- Several methods, including numerical taxonomy and genetic homology, are currently used to determine evolutionary relationships among organisms.

Numerical Taxonomy

- In **numerical taxonomy**, organisms are compared on the basis of a large number of characteristics and grouped according to the percentage of shared characteristics.

Genetic Homology

- **Genetic homology** is the similarity of DNA among different organisms, which provides a measure of their relatedness. Several techniques that determine genetic homology are available.
- The relative percentages of bases in DNA are a measure of relatedness. The base composition of DNA is determined, and G—C percentages are compared among organisms.
- Base sequences can be identified by DNA **probes**.
- In **DNA hybridization**, the degree of matching between strands of DNA is compared among organisms.
- **Protein profiles**, made by **polyacrylamide gel electrophoresis (PAGE)** are used to indicate whether the same proteins are present in different organisms.
- The amino acid sequences of related organisms are similar, so the determination of amino acid sequences is another measure of relatedness.

Other Techniques

- Other methods make use of properties of ribosomes, immunological reactions, and **phage typing**.

The Significance of Findings

Evolutionary relationships can be used to group closely related organisms. Small differences among organisms descended from a common ancestor arise by **divergent evolution**. An early divergence gave rise to the two major subgroups of eubacteria, the Gram-positive bacteria and the Gram-negative ones.

BACTERIAL TAXONOMY AND NOMENCLATURE

Criteria for Classifying Bacteria

- The criteria used for classifying bacteria are summarized in Table 9.4. These criteria can be used to classify bacteria into species and even into strains within species.

- For many species a particular strain is designated as the **type strain**, which is preserved in a type culture collection.

The History and Significance of *Bergey's Manual*

- *Bergey's Manual of Determinative Bacteriology* was first published in 1923 and has been revised several times; a ninth edition was published in 1994.
- *Bergey's Manual of Systematic Bacteriology* (a four-volume set) provides definitive information on the identification and classification of bacteria.

Problems Associated with Bacterial Taxonomy

- Taxonomists do not agree on how members of the kingdom Prokaryotae (Monera) should be divided. Many species of bacteria have been grouped into genera and some into families. Four *divisions* (the equivalent of phyla) have been established. Much information is needed to determine evolutionary relationships and establish classes and orders.

Bacteria by Section of *Bergey's Manual*

- The genera of medical significance, their characteristics, and the diseases they cause are summarized in Table 9.7. A complete listing of the sections is provided in Appendix B.
- Groups of important bacteria include the spirochetes, mycoplasmas, rickettsiae, chlamydiae, mycobacteria, and cyanobacteria.

Bacterial Taxonomy and You

- Use the endpapers at the beginning and end of the book to familiarize yourself with taxonomic information.

TERMINOLOGY CHECK

Animalia ***(p. 239)***
Anneal ***(p. 249)***
Archaea ***(p. 241)***
archaeobacteria ***(p. 238)***
Bacteria ***(p. 241)***
binomial nomenclature ***(p. 233)***
Chlamydiae ***(p. 257)***
cyanobacteria ***(p. 238)***
dichotomous key ***(p. 235)***
divergent evolution ***(p. 251)***
DNA hybridization ***(p. 249)***
domain ***(p. 240)***
eubacteria ***(p. 238)***
Eukarya ***(p. 241)***
eukaryote ***(p. 237)***
extreme halophile ***(p. 244)***
extreme thermoacidophile ***(p. 244)***
five-kingdom system ***(p. 237)***
Fungi ***(p. 239)***
genetic homology ***(p. 248)***
genus ***(p. 233)***
methanogen ***(p. 244)***
Monera ***(p. 237)***
Mycoplasmas ***(p. 257)***
numerical taxonomy ***(p. 247)***
phage typing ***(p. 251)***
phylogenetic ***(p. 235)***
Plantae ***(p. 239)***
polyacrylamide gel electrophoresis (PAGE) ***(p. 250)***
probe ***(p. 249)***
Prokaryotae ***(p. 237)***
prokaryote ***(p. 237)***
protein profile ***(p. 250)***
Protista ***(p. 239)***
Rickettsiae ***(p. 257)***
shrub of life ***(p. 243)***
species ***(p. 233)***
specific epithet ***(p. 233)***
strain ***(p. 234)***
stromatolite ***(p. 240)***
taxon ***(p. 233)***
taxonomy ***(p. 233)***
type strain ***(p. 252)***
Ureaplasmas ***(p. 257)***
virus ***(p. 244)***

CLINICAL CASE STUDY

The following is a true story, though the name has been changed. It is an example of why it is critically important to identify organisms that are causing disease: Dr. Overland had just retired from teaching, she was a diabetic and went to the hospital to have a diagnostic procedure done, called an angiogram. This involves injecting dye in the circulatory system to analyze the function of the heart. A few days after the procedure Dr. Overland began to be feverish. She went to her doctor, who put her in the hospital and began treating her with an antibiotic to kill bacteria. Blood samples were collected to begin identification of the organism that was causing the disease process. The organism turned out not to be a bacterium but a yeastlike fungus, *Candida albicans*. Dr. Overland died before an antimicrobial could be used to kill the *Candida albicans*. Why did it take the hospital so long to identify the pathogen?

CRITICAL THINKING QUESTIONS

1. Before Linnaeus created binomial nomenclature, in which scientific names are uniform worldwide, the same organism had different names in different parts of the world. Is there any reason why that would have presented problems to scientists?

2. Some experts have suggested that the concept of species be replaced with some other system in naming bacteria. Can you think of any reasons why someone might make that suggestion?

3. Every day a clinical laboratory receives specimens containing pathogens that need to be identified. A variety of newer and older methods are commonly used to identify those pathogens. What are some methods now in use that Louis Pasteur would not have understood?

SELF-QUIZ

1. The science involved with the classification of organisms is:
(a) Taxonomy (d) Classificology
(b) Microbiology (e) Scientology
(c) Namology

2. Taxonomy is the science of classification of living things and provides:
(a) A way to identify organisms
(b) Arrangement of related organisms into groups
(c) Information on how organisms have evolved
(d) Only (a) and (b)
(e) (a), (b), and (c) are correct

3. Using binomial nomenclature, the first name designates the genus while the second name designates the:
(a) Specific epithet (d) Kingdom
(b) Order (e) Phylum
(c) Family

4. When a dichotomous key is used to identify organisms:
(a) It consists of two choices than then identify an organism
(b) It uses a flow chart in which there are always two either / or choices for each characteristic
(c) It uses the DNA sequence of organisms to identify them
(d) It uses a set of charts with all the characteristics of organisms
(e) It is the only method to identify bacteria

5. If typed, the genus and species name should be italicized. If written, they should be _____.
(a) Highlighted
(b) Written in all capital letters
(c) Written in bold ink
(d) Underlined
(e) Written in blue ink

6. Members of a species can sometimes be subdivided into subgroups called:
(a) Orders (d) Families
(b) Genera (e) Kingdoms
(c) Strains

7. Which of the following is not a prokaryote?
(a) Bacterium
(b) Paramecium
(c) Blue-green algae
(d) Cyanobacteria
(e) Archaeobacteria

8. In eukaryotes and prokaryotes the ability to successfully mate is used to identify organisms at the species level. True or false?

9. What type of bacteria are also called blue-green algae?
(a) Paramecium
(b) Archaeobacteria
(c) Eubacteria
(d) Protists
(e) Cyanobacteria

10. All of the following pertain to archaeobacteria EXCEPT:
(a) They include microbes that live in hot acidic environments
(b) All are strict anaerobes
(c) They include microbes that live in extremely salty environments
(d) All lack peptidoglycan in their cell walls
(e) They include microbes that reduce carbon to methane gas

11. Members of the kingdom Protista differ from members of the kingdom Monera mainly due to the presence of:
(a) RNA
(b) Ribosomes
(c) Cell wall
(d) DNA
(e) Membrane-bound nucleus

12. Extreme thermophiles grow in conditions of high:
(a) Temperature (d) Nitrogen
(b) Salt (e) Methane
(c) Oxygen

13. Extreme halophiles grow in conditions containing high amounts of:
(a) Nitrogen (d) Oxygen
(b) Temperature (e) Salt
(c) Methane

14. An organism that contains 36% G—C will also contain:
(a) 36% A—T (d) 64% A—T
(b) 36% A + 64%T (e) 36% A + 36%T
(c) 64% A + 36%T

15. In polyacrylamide gel electrophoresis, proteins or nucleic acids of greater size move more _____ than those of smaller size.
(a) Laterally (d) The same
(b) Quickly (e) Diffusely
(c) Slowly

16. DNA encoding which of the following cell components would most likely be conserved throughout evolution of an organism?
(a) Flagella
(b) Ribosomes
(c) Antibiotic resistance
(d) Antigenic proteins
(e) Membrane proteins

17. Which of the following would be the most specific method for classifying bacteria?
(a) DNA analysis (d) Size
(b) Phage typing (e) Capsules
(c) Morphology

18. Mycoplasmas lack what cell structures?
(a) Cell walls (d) DNA
(b) Cell membranes (e) Ribosomes
(c) RNA

19. Small proteins that are capable of causing diseases such as mad cow disease are called what?
(a) Viroids (d) Protons
(b) Prions (e) Bacteriophages
(c) RNA viruses

20. Which of the following references would be most beneficial for identifying an unknown bacterial isolate?
(a) Encyclopedia
(b) Dictionary
(c) *Bergey's Manual of Systematic Bacteriology*
(d) Microbiology textbook
(e) Your microbiology professor

21. A method used to amplify short fragments of DNA is:
(a) DNA hybridization
(b) Southern blotting
(c) Restriction fragment length polymorphism
(d) Cloning
(e) Polymerase chain reaction

22. Which of the following characteristics is used to classify viruses?
(a) Type and arrangement of nucleic acids
(b) Capsid shape
(c) Presence or absence of an envelope
(d) Presence or absence of tail structures
(e) All of these

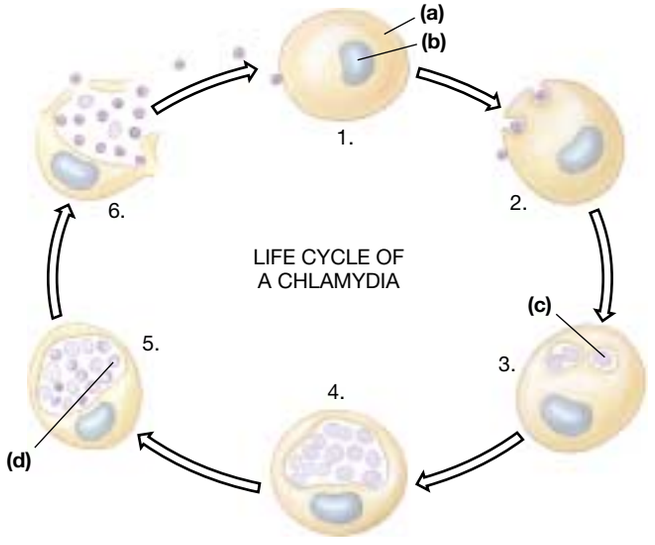
23. Match the following with their respective descriptions:

____ Animalia	(a) Usually unicellular eukaryotes
____ Plantae	(b) Unicellular and multicellular absorptive heterotrophs
____ Protista	(c) Multicellular ingestive heterotrophs
____ Monera	(d) Multicellular and photosynthetic
____ Fungi	(e) Unicellular prokaryotes

24. Microbiologists use which of the following to help them identify a bacterium?
(a) The Internet
(b) Multiple reference texts
(c) *Pasteur's Dictionary of Bacteriology*
(d) *Bergey's Manual of Determinative Bacteriology*
(e) *Black's Manual of Taxonomy*

25. In the name *Escherichia coli*, what is the species name?
(a) *Escherichia*
(b) *coli*
(c) *Escherichia coli*
(d) None of the above

26. In the following diagram of the life cycle of *Chlamydia trachomatis*, identify numbered stages 1–6 and parts (a)–(d).



EXPLORATIONS ON THE WEB

http://www.wiley.com/college/black

If you think you've mastered this chapter, there's more to challenge you on the web. Go to the companion web site to fine-tune your understanding of the chapter concepts and discover answers to the questions posed below.

1. Members of genera belonging to the Enterobacteriaceae family cause such diseases as meningitis, bacillary dysentery, typhoid, and food poisoning. So, if you are going to get infected with an enteric bacteria do you want it to be able to ferment lactose?

2. Which Gram-negative, nonfermenter rod is responsible for many nosocomial infections, especially in immunocompromised individuals, burn victims, and individuals on respirators or with indwelling catheters?

3. Learn more about why scientists now believe there are three rather than two evolutionary lineages.