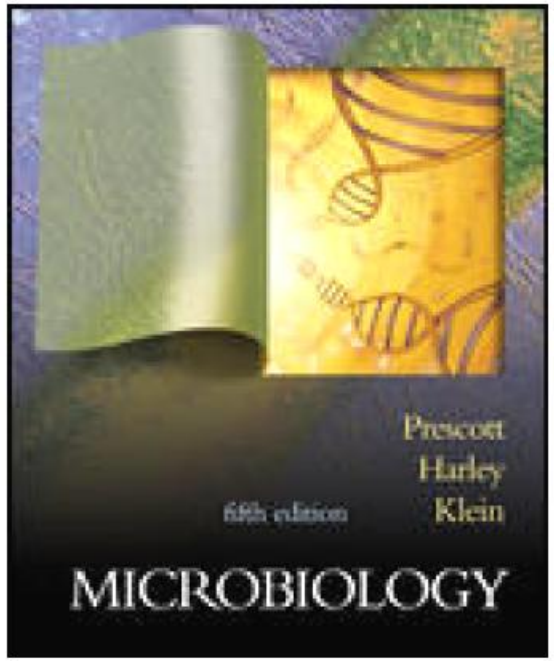
# Evolution and Taxonomy of Microorganisms

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# Microbial Evolution and Diversity

- It has been estimated that our planet is about 4.6 billion years old.
- Fossilized remains of procaryotic cells around 3.5 to 3.8 billion years old have been discovered in stromatolites and sedimentary rocks.
- Thus procaryotic life arose very shortly after the earth cooled.
- Very likely the earliest procaryotes were anaerobic.
- Cyanobacteria and oxygen-producing photosynthesis probably developed 2.5 to 3.0 billion or more years ago.
- Microbial diversity increased greatly as oxygen became more plentiful.
- The studies of Carl Woese and his collaborators on rRNA sequences in procaryotic cells suggest that procaryotes divided into two distinct groups very early on.
- The tree is divided into three major branches representing the three primary groups: *Bacteria, Archaea,* and *Eucarya*.
- The archaea and bacteria first diverged, then the eucaryotes developed.
- These three primary groups are called **domains** and placed above the phylum and kingdom levels

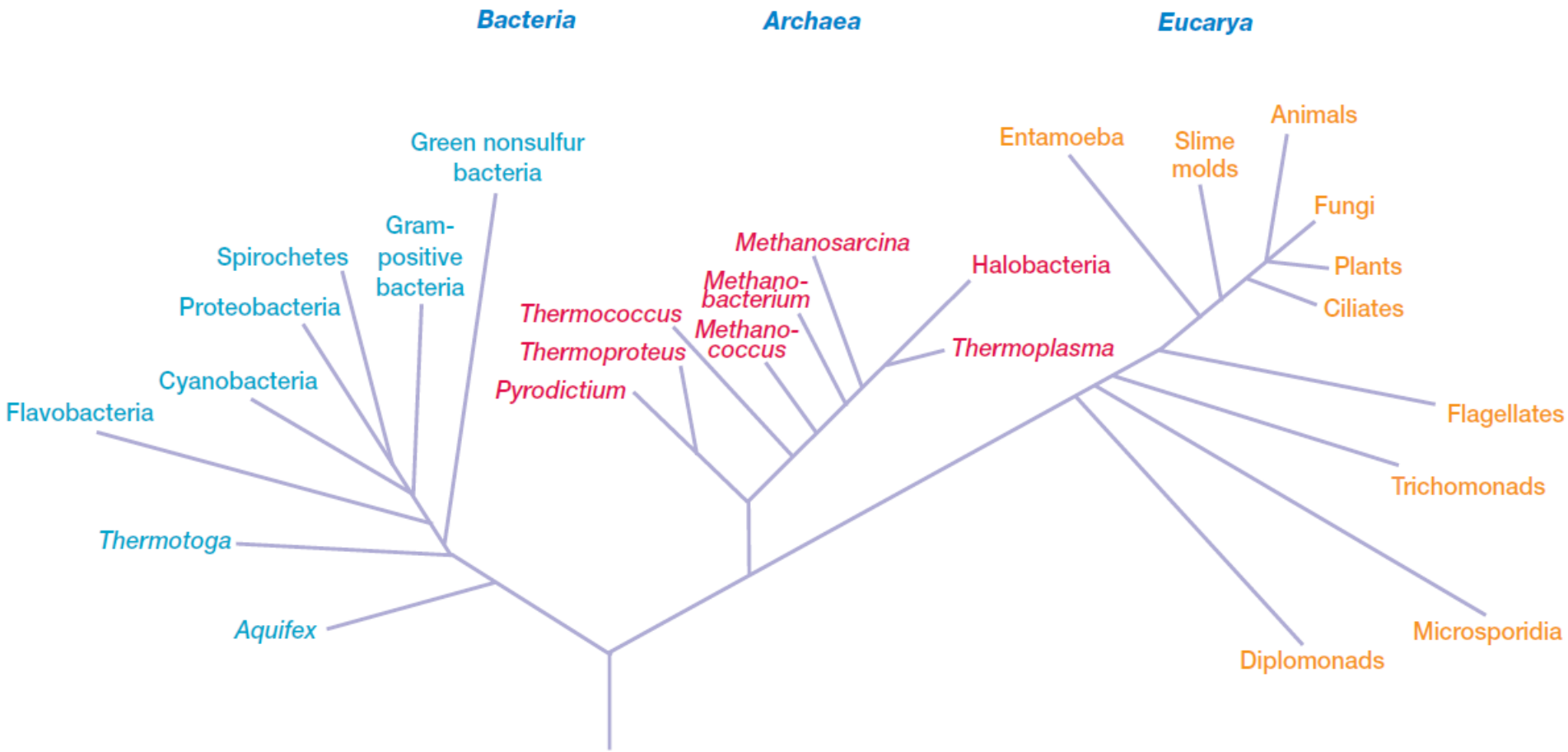


**Figure 19.3 Universal Phylogenetic Tree.** These relationships were determined from rRNA sequence comparisons. *Source: Adapted from G. J. Olsen and C. R. Woese. "Ribosomal RNA: A Key to Phylogeny" in* The FASEB Journal, *7:113–123, 1993.*

# Evolution of Eucaryotic Cells

- It appears likely that modern eucaryotic cells arose from procaryotes about 1.4 billion years ago.
- It is not certain how this process occurred, and two hypotheses have been proposed.
- According to the first, nuclei, mitochondria, and chloroplasts arose by invagination of the plasma membrane to form double-membrane structures containing genetic material and capable of further development and specialization.
- The similarities between chloroplasts, mitochondria, and modern bacteria are due to conservation of primitive procaryotic features by the slowly changing organelles.
- According to the more popular **endosymbiotic hypothesis, the** ancestral eucaryotic cell may have developed from a fusion of ancient bacteria and archaea.
- Possibly a gram-negative bacterial host cell that had lost its cell wall engulfed an archaeon to form an endosymbiotic association.
- The archaeon subsequently lost its wall and plasma membrane, while the host bacterium developed membrane infolds.
- Eventually the host genome was transferred to the original archaeon, and a nucleus and the endoplasmic reticulum was formed.

# Mitochondria and chloroplasts appear to have developed later

- The free-living, fermenting ancestral eucaryote with its nucleus established a permanent symbiotic relationship with photosynthetic bacteria, which then evolved into chloroplasts.
- Cyanobacteria have been considered the most likely ancestors of chloroplasts.
- More recently *Prochloron* (chlorophyte) has become the favorite candidate.
- Mitochondria arose from an endosymbiotic relationship between the free-living primitive eucaryote and bacteria with aerobic respiration.

# Cyanelle

- The endosymbiotic hypothesis has received support from the discovery of an endosymbiotic cyanobacterium that inhabits the biflagellate protist *Cyanophora paradoxa* and acts as its chloroplast.
- This endosymbiont, called a cyanelle, resembles the cyanobacteria in its photosynthetic pigment system and fine structure and it is surrounded by a peptidoglycan layer.
- It differs from cyanobacteria in lacking the lipopolysaccharide outer membrane characteristic of gram-negative bacteria.
- The cyanelle may be a recently established endosymbiont that is evolving into a chloroplast.
- Further support is provided by rRNA trees, which locate chloroplast RNA within the cyanobacteria.

# Taxonomy

- **Taxonomy** is defined as the science of biological classification.
- In a broader sense it consists of three separate but interrelated parts: classification, nomenclature, and identification.
- **Classification** is the arrangement of organisms into groups or **taxa** (s., **taxon**) based on mutual similarity or evolutionary relatedness.
- **Nomenclature** is the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules.
- **Identification** is the practical side of taxonomy, the process of determining that a particular isolate belongs to a recognized taxon.

# Taxonomic Ranks of Procarkotic Taxonomy

- Microbiologists often use informal names in place of formal hierarchical ones.
- Typical examples of such names are purple bacteria, spirochetes, methane-oxidizing bacteria, sulfate-reducing bacteria, and lactic acid bacteria.
- A **procaryotic species** is a collection of strains that share many stable properties and differ significantly from other groups of strains.
- A species (genomospecies) is a collection of strains that have a similar G C composition and 70% or greater similarity as judged by DNA hybridization experiments.
- Strains within a species may differ slightly from one another in many ways.
- **Biovars** are variant procaryotic strains characterized by biochemical or physiological differences, **morphovars** differ morphologically, and **serovars** have distinctive antigenic properties.
- One strain of a species is designated as the **type strain.**
- It is usually one of the first strains studied and often is more fully characterized than other strains; however, it does not have to be the most representative member.

**Table 19.1** An Example of Taxonomic Ranks and Names

| Rank | Example |
|---|---|
| Domain | *Bacteria* |
| Phylum | *Proteobacteria* |
| Class | γ-Proteobacteria |
| Order | *Enterobacteriales* |
| Family | *Enterobacteriaceae* |
| Genus | *Shigella* |
| Species | *S. dysenteriae* |

- Microbiologists name microorganisms by using the **binomial system** of the Swedish botanist Carl von Linné, or Carolus Linnaeus as he often is called.

# Classification Systems

- The most desirable classification system, called a **natural classification,** arranges organisms into groups whose members share many characteristics and reflects as much as possible the biological nature of organisms.
- There are two general ways in which classification systems can be constructed.
- Organisms can be grouped together based on overall similarity to form a **phenetic system** or they can be grouped based on probable evolutionary relationships to produce a **phylogenetic system**.
- Computers may be used to analyze data for the production of phenetic classifications. The process is called **numerical taxonomy**.
- **Phenetic Classification** : phenetic system, one that groups organisms together based on the mutual similarity of their phenotypic characteristics.
- Although phenetic studies can reveal possible evolutionary relationships, they are not dependent on phylogenetic analysis.

# Numerical taxonomy

- The development of computers has made possible the quantitative approach known as **numerical taxonomy.**
- The process begins with a determination of the presence or absence of selected characters in the group of organisms under study.
- A character usually is defined as an attribute about which a single statement can be made.
- It is best to include many different kinds of data: morphological, biochemical, and physiological.
- After character analysis, an association coefficient, a function that measures the agreement between characters possessed by two organisms, is calculated for each pair of organisms in the group.
- The **simple matching coefficient (*SSM*)**, the most commonly used coefficient in bacteriology, is the proportion of characters that match regardless of whether the attribute is present or absent.

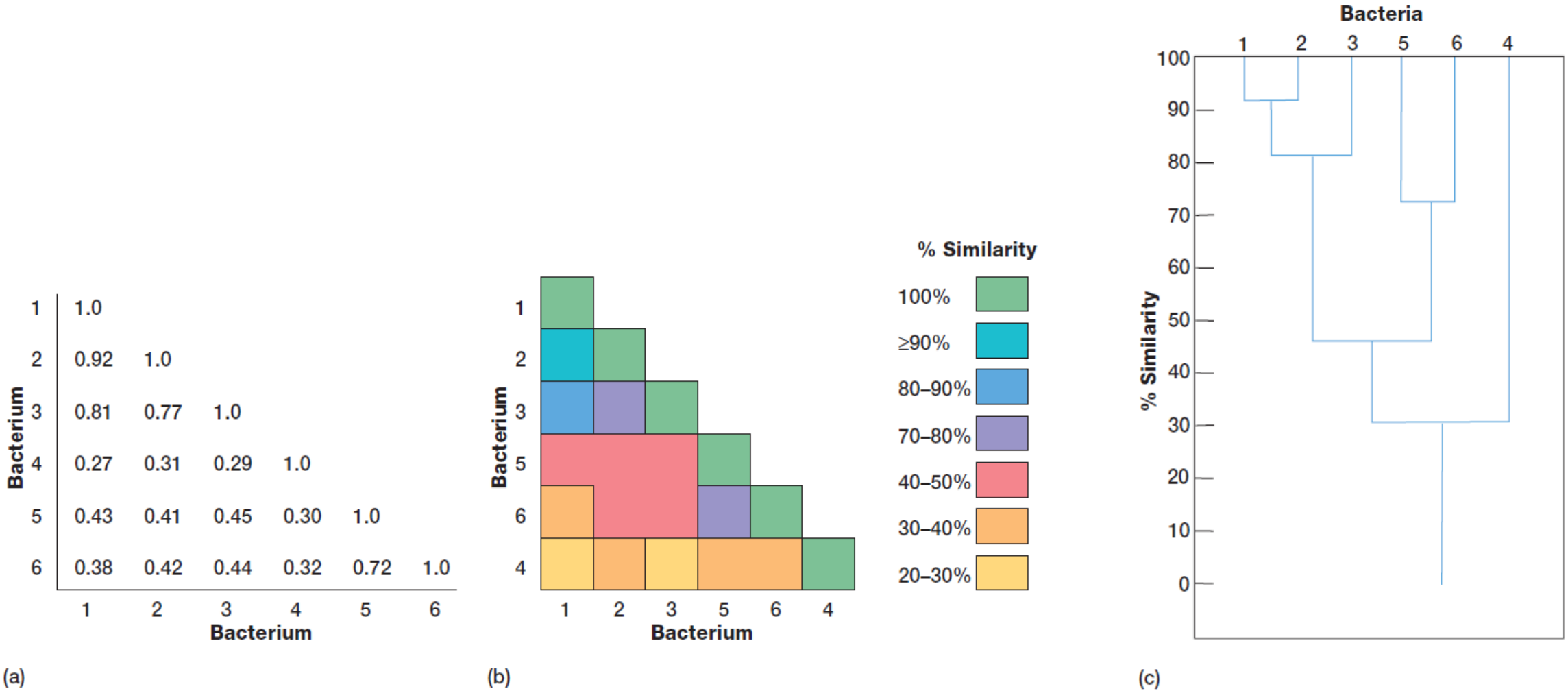


**Figure 19.5 Clustering and Dendrograms in Numerical Taxonomy.** (**a**) A small similarity matrix that compares six strains of bacteria. The degree of similarity ranges from none (0.0) to complete similarity (1.0). (**b**) The bacteria have been rearranged and joined to form clusters of similar strains. For example, strains 1 and 2 are the most similar. The cluster of 1 plus 2 is fairly similar to strain 3, but not at all to strain 4. (**c**) A dendrogram showing the results of the analysis in part *b*. Strains 1 and 2 are members of a 90-phenon, and strains 1–3 form an 80-phenon. While strains 1–3 may be members of a single species, it is quite unlikely that strains 4–6 belong to the same species as 1–3.

# Phylogenetic Classification

- These are systems based on evolutionary relationships rather than general resemblance (the term **phylogeny** [Greek *phylon,* tribe or race, and *genesis,* generation or origin] refers to the evolutionary development of a species).
- This has proven difficult for procaryotes and other microorganisms, primarily because of the lack of a good fossil record.
- The direct comparison of genetic material and gene products such as RNA and proteins overcomes many of these problems.

# Major Characteristics Used in Taxonomy

- For sake of clarity, characteristics have been divided into two groups: classical and molecular.
- **Classical:** Classical approaches to taxonomy make use of morphological, physiological, biochemical, ecological, and genetic characteristics.
- ***Morphological Characteristics***
- Morphology is easy to study and analyze, particularly in eucaryotic microorganisms and the more complex procaryotes.

**Table 19.3** Some Morphological Features Used in Classification and Identification

| Feature | Microbial Groups |
|---|---|
| Cell shape | All major groups[a] |
| Cell size | All major groups |
| Colonial morphology | All major groups |
| Ultrastructural characteristics | All major groups |
| Staining behavior | Bacteria, some fungi |
| Cilia and flagella | All major groups |
| Mechanism of motility | Gliding bacteria, spirochetes |
| Endospore shape and location | Endospore-forming bacteria |
| Spore morphology and location | Bacteria, algae, fungi |
| Cellular inclusions | All major groups |
| Color | All major groups |

# Physiological and Metabolic Characteristics

- Physiological and metabolic characteristics are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins.
- Since proteins are gene products, analysis of these characteristics provides an indirect comparison of microbial genomes.

**Table 19.4** Some Physiological and Metabolic Characteristics Used in Classification and Identification

| |
|---|
| Carbon and nitrogen sources |
| Cell wall constituents |
| Energy sources |
| Fermentation products |
| General nutritional type |
| Growth temperature optimum and range |
| Luminescence |
| Mechanisms of energy conversion |
| Motility |
| Osmotic tolerance |
| Oxygen relationships |
| pH optimum and growth range |
| Photosynthetic pigments |
| Salt requirements and tolerance |
| Secondary metabolites formed |
| Sensitivity to metabolic inhibitors and antibiotics |
| Storage inclusions |

# Ecological Characteristics

- Even very closely related microorganisms can differ considerably with respect to ecological characteristics.
- Microorganisms living in various parts of the human body markedly differ from one another and from those growing in freshwater, terrestrial, and marine environments.

**Genetic Analysis**

- Because most eucaryotes are able to reproduce sexually, genetic analysis has been of considerable usefulness in the classification of these organisms.
- The study of chromosomal gene exchange through transformation and conjugation is sometimes useful in bacterial classification.

# Molecular Characteristics

- Some of the most powerful approaches to taxonomy are through the study of proteins and nucleic acids.
- ***Comparison of Proteins***
- The amino acid sequences of proteins are direct reflections of mRNA sequences and therefore closely related to the structures of the genes coding for their synthesis.
- For this reason, comparisons of proteins from different microorganisms are very useful taxonomically.
- There are several ways to compare proteins.
- The most direct approach is to determine the amino acid sequence of proteins with the same function.
- If the sequences of proteins with the same function are similar, the organisms possessing them are probably closely related.
- The sequences of cytochromes and other electron transport proteins, histones, heat-shock proteins, transcription and translation proteins, and avariety of metabolic enzymes have been used in taxonomic studies.

# Nucleic Acid Base Composition

- In double-stranded DNA, A pairs with T, and G pairs with C.
- Thus the (G + C)/(A+T) ratio or **G + C content,** the percent of G + C in DNA, reflects the base sequence and varies with sequence changes as follows:

$$\text{Mol\% G + C} = \frac{\text{G + C}}{\text{G + C + A + T}} \times 100$$

- The base composition of DNA can be determined in several ways.
- Although the G + C content can be ascertained after hydrolysis of DNA and analysis of its bases with high-performance liquid chromatography (HPLC), physical methods are easier and more often used.
- The G + C content often is determined from the **melting temperature (*Tm*)** of DNA.
- DNA with a greater G + C content will have more hydrogen bonds, and its strands will separate only at higher temperatures—that is, it will have a higher melting point.
- DNA melting can be easily followed spectrophotometrically because the absorbance of 260 nm UV light by DNA increases during strand separation.
- The midpoint of the rising curve gives the melting temperature, a direct measure of the G+ C content.

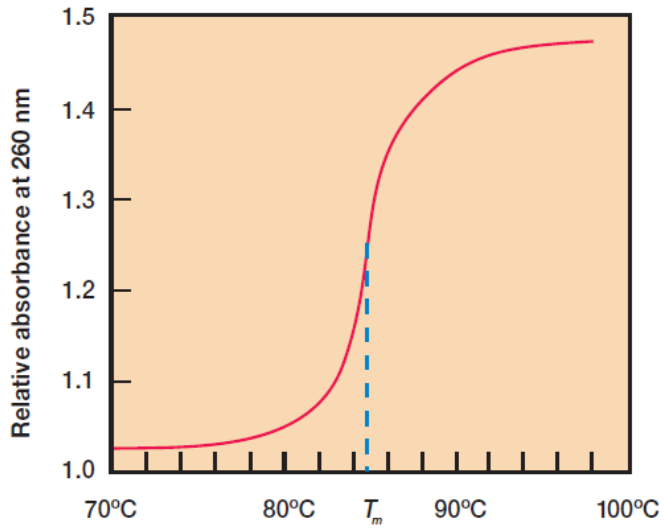


**Figure 19.6** **A DNA Melting Curve.** The $T_m$ is indicated.

**Table 19.5** Representative G + C Contents of Microorganisms

| Organism | Percent G + C |
|---|---|
| **Bacteria** | |
| *Actinomyces* | 59–73 |
| *Anabaena* | 38–44 |
| *Bacillus* | 32–62 |
| *Bacteroides* | 28–61 |
| *Bdellovibrio* | 33–52 |
| *Caulobacter* | 63–67 |
| *Chlamydia* | 41–44 |
| *Chlorobium* | 49–58 |
| *Chromatium* | 48–70 |
| *Clostridium* | 21–54 |
| *Cytophaga* | 33–42 |
| *Deinococcus* | 62–70 |
| *Escherichia* | 48–52 |
| *Halobacterium* | 66–68 |
| *Hyphomicrobium* | 59–67 |
| *Methanobacterium* | 32–50 |
| *Micrococcus* | 64–75 |
| *Mycobacterium* | 62–70 |
| *Mycoplasma* | 23–40 |
| *Myxococcus* | 68–71 |
| *Neisseria* | 47–54 |
| *Nitrobacter* | 60–62 |
| *Oscillatoria* | 40–50 |
| *Prochloron* | 41 |
| *Proteus* | 38–41 |
| *Pseudomonas* | 58–70 |
| *Rhodospirillum* | 62–66 |
| *Rickettsia* | 29–33 |
| *Salmonella* | 50–53 |
| *Spirillum* | 38 |
| *Spirochaeta* | 51–65 |
| *Staphylococcus* | 30–38 |
| *Streptococcus* | 33–44 |
| *Streptomyces* | 69–73 |
| *Sulfolobus* | 31–37 |
| *Thermoplasma* | 46 |
| *Thiobacillus* | 52–68 |
| *Treponema* | 25–54 |
| **Algae** | |
| *Acetabularia mediterranea* | 37–53 |
| *Chlamydomonas* | 60–68 |
| *Chlorella* | 43–79 |
| *Cyclotella cryptica* | 41 |
| *Euglena gracilis* | 46–55 |
| *Nitella* | 49 |
| *Nitzschia angularis* | 47 |
| *Ochromonas danica* | 48 |
| *Peridinium triquetrum* | 53 |
| *Scenedesmus* | 52–64 |
| *Spirogyra* | 39 |
| *Volvox carteri* | 50 |
| **Protozoa** | |
| *Acanthamoeba castellanii* | 56–58 |
| *Amoeba proteus* | 66 |
| *Paramecium* spp. | 29–39 |
| *Plasmodium berghei* | 41 |
| *Stentor polymorphus* | 45 |
| *Tetrahymena* | 19–33 |
| *Trichomonas* | 29–34 |
| *Trypanosoma* | 45–59 |
| **Slime Molds** | |
| *Dictyostelium* | 22–25 |
| *Lycogala* | 42 |
| *Physarum polycephalum* | 38–42 |
| **Fungi** | |
| *Agaricus bisporus* | 44 |
| *Amanita muscaria* | 57 |
| *Aspergillus niger* | 52 |
| *Blastocladiella emersonii* | 66 |
| *Candida albicans* | 33–35 |
| *Claviceps purpurea* | 53 |
| *Coprinus lagopus* | 52–53 |
| *Fomes fraxineus* | 56 |
| *Mucor rouxii* | 38 |
| *Neurospora crassa* | 52–54 |
| *Penicillium notatum* | 52 |
| *Polyporus palustris* | 56 |
| *Rhizopus nigricans* | 47 |
| *Saccharomyces cerevisiae* | 36–42 |
| *Saprolegnia parasitica* | 61 |

# Nucleic Acid Hybridization

- The similarity between genomes can be compared more directly by use of **nucleic acid hybridization** studies.
- If a mixture of single stranded DNA formed by heating dsDNA is cooled and held at a temperature about 25°C below the *Tm*, strands with complementary base sequences will reassociate to form stable dsDNA, whereas noncomplementary strands will remain single (**figure 19.7**).
- Because strands with similar, but not identical, sequences associate to form less temperature stable dsDNA hybrids, incubation of the mixture at 30 to 50°C below the *Tm* will allow hybrids of more diverse ssDNAs to form.
- Incubation at 10 to 15°C below the *Tm* permits hybrid formation only with almost identical strands.
- In one of the more widely used hybridization techniques, nitrocellulose filters with bound nonradioactive DNA strands are incubated at the appropriate temperature with single-stranded DNA fragments made radioactive with 32P, 3H, or 14C.
- After radioactive fragments are allowed to hybridize with the membrane-bound ss-DNA, the membrane is washed to remove any nonhybridized ssDNA and its radioactivity is measured.
- The quantity of radioactivity bound to the filter reflects the amount of hybridization and thus the similarity of the DNA sequences.
- The degree of similarity or homology is expressed as the percent of experimental DNA radioactivity retained on the filter compared with the percent of homologous DNA radioactivity bound under the same conditions.
- Two strains whose DNAs show at least 70% relatedness under optimal hybridization conditions and less than a 5% difference in *Tm* often are considered members of the same species.

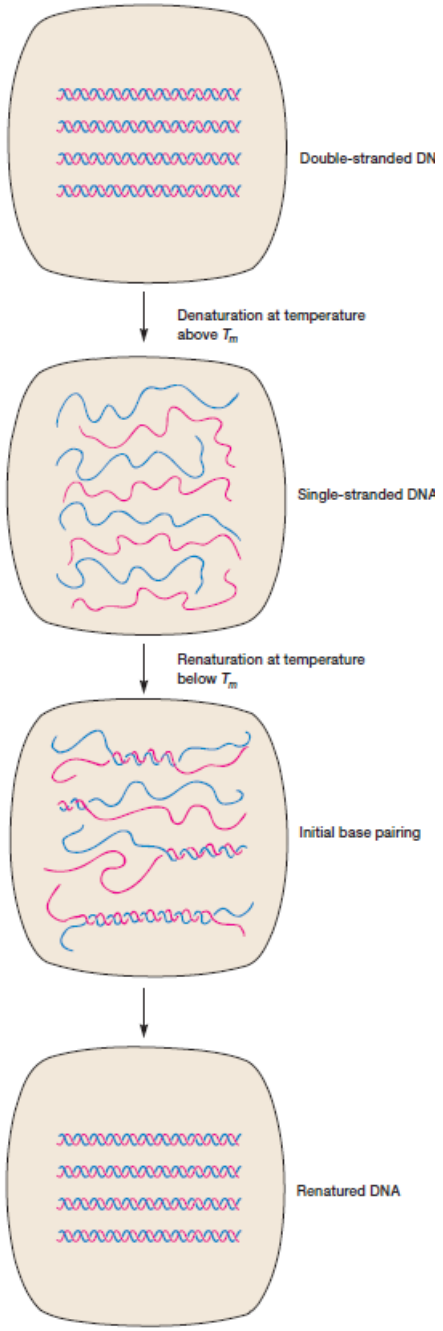


**Figure 19.7 Nucleic Acid Melting and Hybridization.**
Complementary strands are shown in different colors.

# Nucleic Acid Sequencing

- Most attention has been given to sequences of the 5S and 16S rRNAs isolated from the 50S and 30S subunits, respectively, of procaryotic ribosomes.
- The rRNAs are almost ideal for studies of microbial evolution and relatedness since they are essential to a critical organelle found in all microorganisms.
- Their functional role is the same in all ribosomes. Furthermore, their structure changes very slowly with time, presumably because of their constant and critical role.
- Because rRNA contains variable and stable sequences, both closely related and very distantly related microorganisms can be compared.
- This is an important advantage as distantly related organisms can be studied only using sequences that change little with time.

# Assessing Microbial Phylogeny

- Phylogenetic relationships are illustrated in the form of branched diagrams or trees.
- A **phylogenetic tree** is a graph made of branches that connect nodes.
- The nodes represent taxonomic units such as species or genes; the external nodes, those at the end of the branches, represent living organisms.
- The tree may have a time scale, or the length of the branches may represent the number of molecular changes that have taken place between the two nodes.
- Finally, a tree may be unrooted or rooted.
- An unrooted tree (figure 19.8*a*) simply represents phylogenetic relationships but does not provide an evolutionary path.
- Figure 19.8*a* shows that A is more closely related to C than it is to either B or D, but does not specify the common ancestor for the four species or the direction of change.
- In contrast, the rooted tree (figure 19.8*b*) does give a node that serves as the common ancestor and shows the development of the four species from this root.

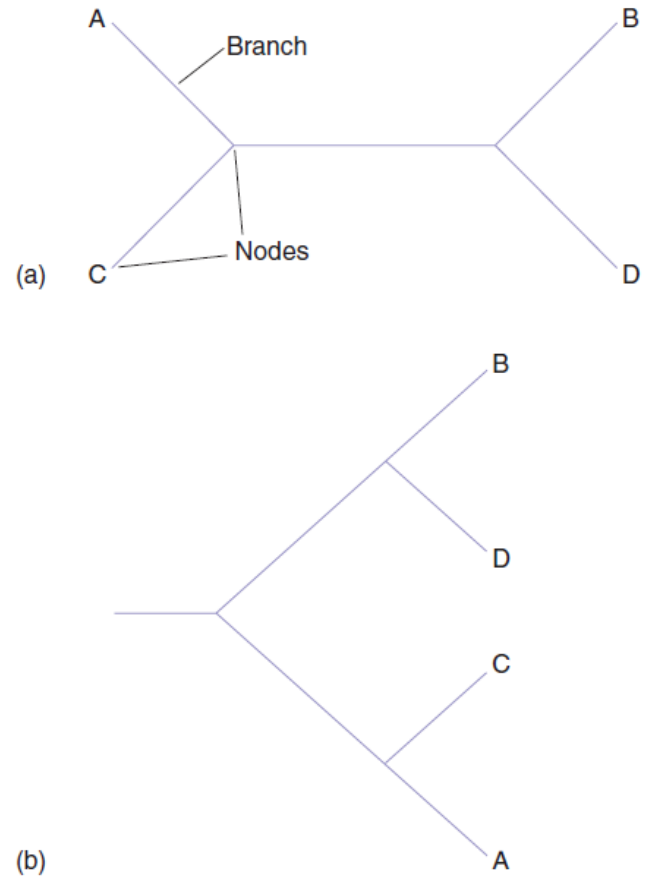


**Figure 19.8 Examples of Phylogenetic Trees.** (a) Unrooted tree joining four taxonomic units. (b) Rooted tree. See text for details.

# …Assessing Microbial Phylogeny

- Phylogenetic trees are developed by comparing molecular sequences.
- To compare two molecules their sequences must first be aligned so that similar parts match up.
- The object is to align and compare homologous sequences, ones that are similar because they had a common origin in the past.
- This is not an easy task, and computers plus fairly complex mathematics must be employed to minimize the number of gaps and mismatches in the sequences being compared.
- **rRNA, DNA, and Proteins as Indicators of Phylogeny**
- Although a variety of molecular techniques are used in estimating the phylogenetic relatedness of procaryotes, the comparison of 16S rRNAs isolated from thousands of strains is of particular important.

# Polyphasic Taxonomy

- Because phylogenetic results vary with the data used in analysis, many taxonomists believe that all possible valid data should be employed in determining phylogeny.
- In the approach called **polyphasic taxonomy,** taxonomic schemes are developed using a wide range of phenotypic and genotypic information ranging from molecular properties to ecological characteristics.
- The techniques that are appropriate for grouping organisms depend on the level of taxonomic resolution needed.
- For example, serological techniques can be used to identify strains, but not genera or species.