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Chapter 19

Microbial Taxonomy

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General Introduction and Overview

- **taxonomy**
  - **science of biological classification**
  - **consists of three separate but interrelated parts**
    - **classification – arrangement of organisms into groups (taxa; s.,taxon)**
    - **nomenclature – assignment of names to taxa**
    - **identification – determination of taxon to which an isolate belongs**

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Importance of taxonomy

- **allows scientists to organize huge amounts of knowledge**
- **allows scientists to make predictions and frame hypotheses about organisms**
- **places organisms into meaningful, useful groups, with precise names, thus facilitating scientific communication**
- **essential for accurate identification of organisms**

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Systematics

- **study of organisms with the ultimate object of characterizing and arranging them in an orderly manner**

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Microbial Evolution and Diversity

- **Earth formed ~ 4.6 billion years ago (bya)**
- **life began to arise soon after planet cooled**

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Appearance of life

- **first procaryotes probably arose at least 3.5 to 3.8 bya**
  - **what appear to be fossilized remains found in stromatolites and sedimentary rocks**
    - **stromatolites – layered rocks formed by incorporation of mineral sediments into microbial mats**
  - **were probably anaerobic**

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Evolution of procaryotes

- **current theories based largely on characterization of rRNA sequences**
  - **work of Carl Woese et al. in 1970s**
- **divided into two distinct groups early on**
  - ***Bacteria***
  - ***Archaea***
- **cyanobacteria (oxygenic phototrophs) arose ~2.5 to 3.0 bya**

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Evolution of eucaryotes

- **arose from procaryotes ~ 1.4 bya**
- **two major hypotheses**
  - **nuclei, mitochondria, and chloroplasts arose by invagination of plasma membranes**
  - **endosymbiotic hypothesis**
    - **arose from a fusion of ancient bacteria and archaea**
    - **chloroplasts arose from free-living phototrophic bacterium that entered symbiotic relationships with primitive eucaryotes**
    - **mitochondria arose by similar mechanism**

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procaryotic, archaeal rRNA, isoprenoid glycerol diether or diglycerol tetraether lipids

eucaryotic, eucaryotic rRNA, diacyl glycerol diester lipids

procaryotic, bacterial rRNA, diacyl glycerol diester lipids

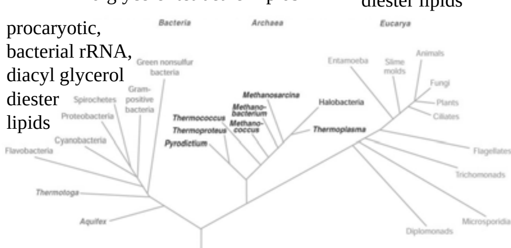

Figure 19.3

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Taxonomic Ranks

- **microbiologists often use informal names**
  - **e.g., purple bacteria, spirochetes, methane-oxidizing bacteria**

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*

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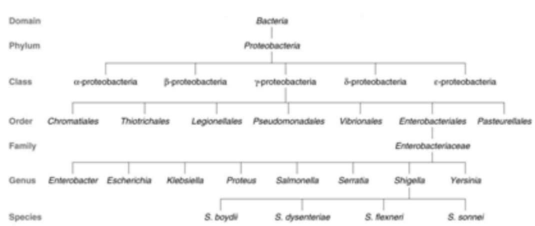

Figure 19.4

genus – well defined group of one or more species that is clearly separate from other genera

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Defining procaryotic species

- **can’t use definition based on interbreeding because procaryotes are asexual**
- **possible definitions:**
  - **collection of strains that share many stable properties and differ significantly from other groups of strains**
  - **collection of strains with similar G + C composition and ≥ 70% sequence similarity**
  - **collection of organisms that share the same sequences in their core housekeeping genes**

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Strains

- **population of organisms that is distinguishable from others within a taxon**
- **descended from a single organism or pure culture isolate**
- **vary from each other in many ways**
  - **biovars – differ biochemically and physiologically**
  - **morphovars – differ morphologically**
  - **serovars – differ in antigenic properties**

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Type strain

- **usually one of first strains of a species studied**
- **often most fully characterized**
- **not necessarily most representative member of species**

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Binomial system of nomenclature

- **devised by Carl von Linné (Carolus Linnaeus)**
- **each organism has two names**
  - **genus name – italicized and capitalized (e.g., *Escherichia*)**
  - **species epithet – italicized but not capitalized (e.g., *coli*)**
- **can be abbreviated after first use (e.g., *E. coli*)**

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Classification Systems

- **natural classification**
  - **arranges organisms into groups whose members share many characteristics**
  - **most desirable system because reflects biological nature of organisms**
- **two methods for construction**
  - **phenetically**
    - **grouped together based on overall similarity**
  - **phylogenetically**
    - **grouped based on probable evolutionary relationships**

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Phenetic Classification

- **groups organisms together based on mutual similarity of phenotypes**
- **can reveal evolutionary relationships, but not dependent on phylogenetic analysis**
  - **i.e., doesn't weight characters**
- **best systems compare as many attributes as possible**

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Numerical Taxonomy

- **used to create phenetic classification systems**
- **multistep process**
  - **code information about properties of organisms**
    - **e.g., 1 = has trait; 0 = doesn't have trait**
  - **use computer to compare organisms on ≥ 50 characters**
  - **determine association coefficient**
  - **construct similarity matrix**
  - **identify phenons and construct dendograms**

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Association coefficients

- **simple matching coefficient**
  - **proportion of characters that match regardless whether attribute is present or absent**
- **Jaccard coefficient**
  - **ignores characters that both lack**

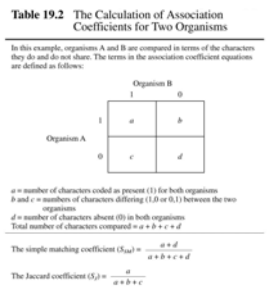

Table 19.2 The Calculation of Association Coefficients for Two Organisms

In this example, organisms A and B are compared in terms of the characters they do and do not share. The terms in the association coefficient equations are defined as follows:

		Organism B 1	Organism B 0
Organism A	1	*a*	*b*
	0	*c*	*d*

a = number of characters coded as present (1) for both organisms
b and c = numbers of characters differing (1,0 or 0,1) between the two organisms
d = number of characters absent (0) in both organisms
Total number of characters compared = $a + b + c + d$

The simple matching coefficient $(S_{SM}) = \frac{a+d}{a+b+c+d}$

The Jaccard coefficient $(S_J) = \frac{a}{a+b+c}$

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- dendogram – treelike diagram used to display results
- phenon – group of organisms with great similarity
  - phenons with ≥80% similarity = bacterial species

similarity matrix → rearranged and joined to show clusters → dendogram

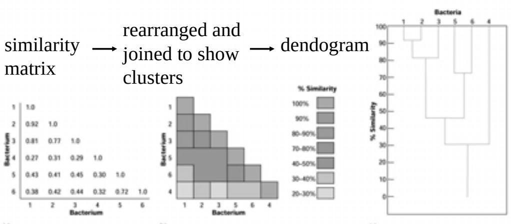

Figure 19.5

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Phylogenetic Classification

- **also called phyletic classification systems**
- **phylogeny**
  - **evolutionary development of a species**
- **usually based on direct comparison of genetic material and gene products**

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Major Characteristics Used in Taxonomy

- two major types
  - classical characteristics
  - molecular characteristics

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Classical Characteristics

- morphological
- physiological and metabolic
- ecological
- genetic analysis

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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

[a]Used in classifying and identifying at least some bacteria, algae, fungi, and protozoa.

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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

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Ecological characteristics

- **life-cycle patterns**
- **symbiotic relationships**
- **ability to cause disease**
- **habitat preferences**
- **growth requirements**

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Genetic analysis

- **study of chromosomal gene exchange by transformation and conjugation**
  - **these processes rarely cross genera**
- **plasmid-borne traits can introduce errors into analysis**

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Molecular Characteristics

- comparison of proteins
- nucleic acid base composition
- nucleic acid hybridization
- nucleic acid sequencing

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Comparison of proteins

- determination of amino acid sequence
- comparison of electrophoretic mobility
- determination of immunological cross-reactivity
- comparison of enzymatic properties

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Nucleic acid base composition

- G + C content
  - Mol% G + C =

    (G + C/G + C + A + T)100
  - usually determined from melting temperature (T_m)
  - variation within a genus usually < 10%

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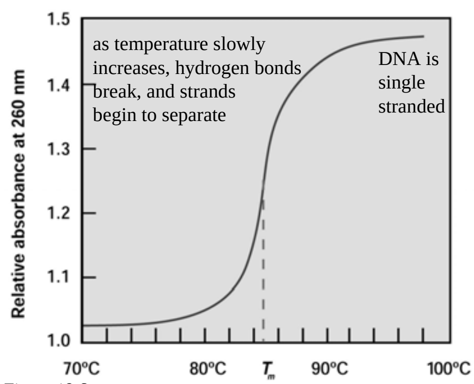

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Table 19.5 Representative G + C Contents of Microorganisms

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

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Nucleic acid hybridization

- **measure of sequence homology**
- **common procedure**
  - **bind nonradioactive DNA to nitrocellulose filter**
  - **incubate filter with radioactive single-stranded DNA**
  - **measure amount of radioactive DNA attached to filter**

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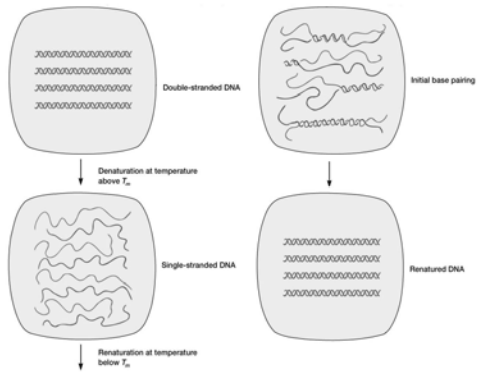

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Table 19.6 Comparison of *Neisseria* Species by DNA Hybridization Experiments

Membrane-Attached DNA[a]	Percent Homology[b]
Neisseria meningitidis	100
N. gonorrhoeae	78
N. sicca	45
N. flava	35

Source: Data from T. E. Staley and R. R. Colwell, "Applications of Molecular Genetics and Numerical Taxonomy to the Classification of Bacteria" in *Annual Review of Ecology and Systematics,* 8: 282, 1973.

[a] The experimental membrane-attached nonradioactive DNA from each species was incubated with radioactive *N. meningitidis* DNA, and the amount of radioactivity bound to the membrane was measured. The more radioactivity bound, the greater the homology between DNA sequences.

[b] $\frac{\textit{N. meningitidis}\text{ DNA bound to experimental DNA}}{\text{Amount bound to membrane-attached }\textit{N. meningitidis}\text{ DNA}} \times 100$

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Nucleic acid sequencing

- **usually comparison of rRNA genes**
- **increasingly, comparison of entire genomes**

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Assessing Microbial Phylogeny

- identify molecular chronometers or other characteristics to use in comparisons of organisms
- illustrate evolutionary relationships in phylogenetic tree

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Molecular Chronometers

- nucleic acids or proteins used as "clocks" to measure amount of evolutionary change over time
- use based on several assumptions
  - sequences gradually change over time
  - changes are selectively neutral and relatively random
  - amount of change increases linearly with time

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Problems with molecular chronometers

- rate of sequence change can vary over time
- different molecules and different parts of molecules can change at different rates

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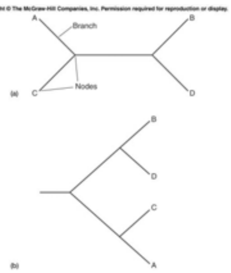
Phylogenetic Trees

nodes = taxonomic units (e.g., species or genes)

terminal nodes = living organisms



rooted tree – has node that serves as common ancestor

Figure 19.8

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Creating phylogenetic trees from molecular data

- **align sequences**
- **determine number of positions that are different**
- **express difference**
  - **e.g., evolutionary distance**
- **use measure of difference to create tree**
  - **organisms clustered based on relatedness**
  - **parsimony – fewest changes from ancestor to organism in question**

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rRNA, DNA, and Proteins as Indicators of Phylogeny

- **all are used**
- **do not always produce the same phylogenetic trees**

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Small subunit rRNA

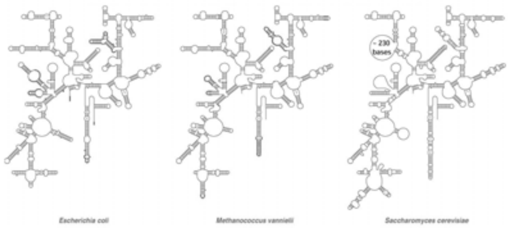

Figure 19.9

frequently used to create trees showing broad relationships

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oligonucleotide signature sequences – specific sequences that occur in most or all members of a phylo-genetic group

useful for placing organisms into kingdom or domain

Table 19.7 Selected 16S rRNA Signature Sequences for Some Bacterial Groups[a]

Position in rRNA	Consensus Composition	γ-Proteobacteria	Cyanobacteria	Spirochetes	*Bacteroides*	Green Sulfur	Green Nonsulfur	*Deinococcus*	Gram Positive (Low GC)	Gram Positive (High GC)	*Planctomyces*
47	C	+	+	U	+	+	+	+	+	+	G
53	A	+	+	G	+	+	G	+	+	+	G
570	G	+	+	+	U	+	+	+	+	+	U
812	G	c	+	+	+	+	+	C	+	+	+
906	G	Ag	+	+	+	+	A	+	+	A	+
955	U	+	+	+	+	+	+	+	+	AC	C
1,207	G	+	C	+	+	+	+	+	C	C	+
1,234	C	+	+	a	U	A	+	+	+	+	+

[a]A plus sign in a column means that the group has the same base as the consensus sequence. If the letter is given in upper case, it is changed in more than 90% of the cases. A lowercase letter signifies a minor occurrence base (<15% of the cases).

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DNA and proteins

- **DNA**
  - **most effective for comparing organisms at species and genus level**
- **proteins**
  - **less affected by organism-specific differences in G + C content**
  - **easier to do sequence alignment**
  - **proteins evolve at different rates**

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Polyphasic Taxonomy

- **use of all possible data to determine phylogeny**
  - **i.e., genotypic and phenotypic information**
- **data used depends on desired level of resolution**
  - **e.g., serological data – resolve strains**
  - **e.g., protein electrophoretic patterns – resolve species**
  - **e.g., DNA hybridization and % G + C – resolve at genus and species level**

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The Major Divisions of Life

- **based primarily on rRNA analysis**
- **currently held that there are three domains of life**
  - ***Bacteria***
  - ***Archaea***
  - ***Eucarya***

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Table 19.8 Comparison of *Bacteria*, *Archaea*, and *Eucarya*

Property	*Bacteria*	*Archaea*	*Eucarya*
Membrane-Enclosed Nucleus with Nucleolus	Absent	Absent	Present
Complex Internal Membranous Organelles	Absent	Absent	Present
Cell Wall	Almost always have peptidoglycan containing muramic acid	Variety of types, no muramic acid	No muramic acid
Membrane Lipid	Have ester-linked, straight-chained fatty acids	Have ether-linked, branched aliphatic chains	Have ester-linked, straight-chained fatty acids
Gas Vesicles	Present	Present	Absent
Transfer RNA	Thymine present in most tRNAs	No thymine in T or TψC arm of tRNA	Thymine present
	N-formylmethionine carried by initiator tRNA	Methionine carried by initiator tRNA	Methionine carried by initiator tRNA
Polycistronic mRNA	Present	Present	Absent
mRNA Introns	Absent	Absent	Present
mRNA Splicing, Capping, and Poly A Tailing	Absent	Absent	Present
Ribosomes			
Size	70S	70S	80S (cytoplasmic ribosomes)
Elongation factor 2	Does not react with diphtheria toxin	Reacts	Reacts
Sensitivity to chloramphenicol and kanamycin	Sensitive	Insensitive	Insensitive
Sensitivity to anisomycin	Insensitive	Sensitive	Sensitive
DNA-Dependent RNA Polymerase			
Number of enzymes	One	Several	Three
Structure	Simple subunit pattern (4 subunits)	Complex subunit pattern similar to eucaryotic enzymes (8–12 subunits)	Complex subunit pattern (12–14 subunits)
Rifampicin sensitivity	Sensitive	Insensitive	Insensitive
Polymerase II Type Promoters	Absent	Present	Present
Metabolism			
Similar ATPase	No	Yes	Yes
Methanogenesis	Absent	Present	Absent
Nitrogen fixation	Present	Present	Absent
Chlorophyll-based photosynthesis	Present	Absent	Present[a]
Chemolithotrophy	Present	Present	Absent

a Present in chloroplasts (of bacterial origin).

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Other possible trees

insert Figure 19.10

Figure 19.10

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Impact of horizontal transfer

- **extensive horizontal gene transfer has occurred within and between domains**
- **pattern of microbial evolution is not as linear and treelike as once thought**

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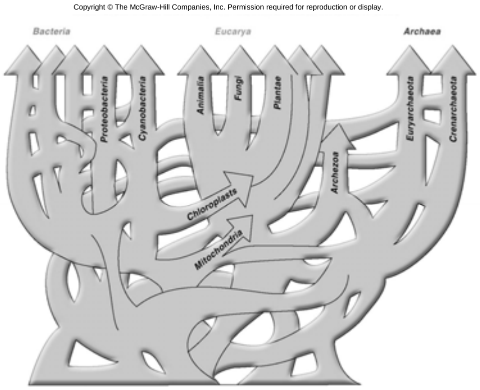

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Kingdoms

multicellular and unicellular, walled eucaryotic cells, absorptive nutrition

multicellular, walled eucaryotic cells, photoautotrophs

unicellular eucaryotes, varied types of nutrition

all procaryotes

multi-cellular, wall-less eucaryotic cells, ingestive nutrition

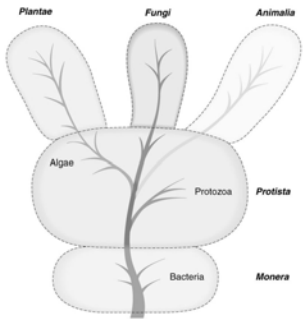

Figure 19.12a

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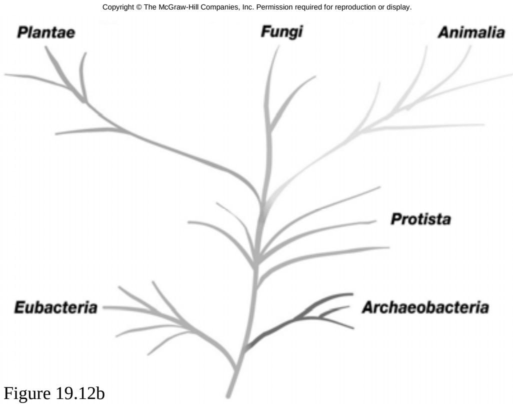

Figure 19.12b

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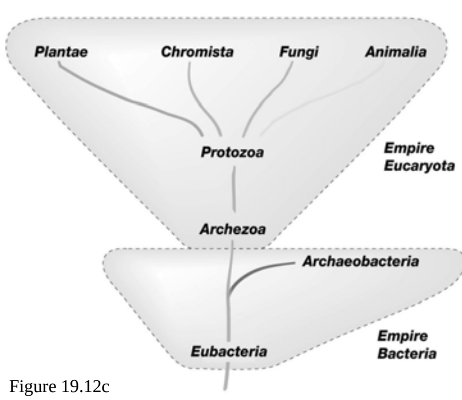

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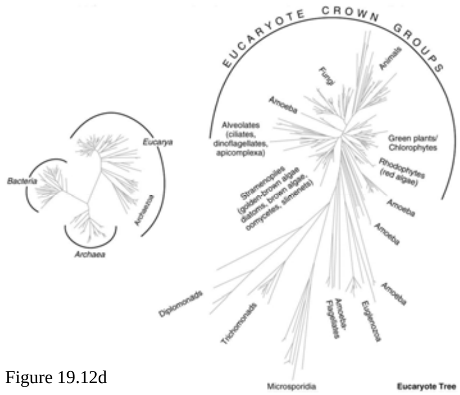

Figure 19.12d

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Bergey's Manual of Systematic Bacteriology

- **detailed work containing descriptions of all procaryotic species currently identified**

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The First Edition of *Bergey's Manual of Systematic Bacteriology*

- **primarily phenetic**
- **cell wall characteristics play important role**

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Table 19.9 Some Characteristic Differences between Gram-Negative and Gram-Positive Bacteria

Property	Gram-negative Bacteria	Gram-positive Bacteria	Mycoplasmas
Cell wall	Gram-negative type wall with inner 2–7 nm peptidoglycan layer and outer membrane (7–8 nm thick) of lipid, protein, and lipopolysaccharide. (There may be a third outermost layer of protein.)	Gram-positive type wall with a homogeneous, thick cell wall (20–80 nm) composed mainly of peptidoglycan. Other polysaccharides and teichoic acids may be present.	Lack a cell wall and peptidoglycan precursors; enclosed by a plasma membrane
Cell shape	Spheres, ovals, straight or curved rods, helices or filaments; some have sheaths or capsules.	Spheres, rods, or filaments; may show true branching	Pleomorphic in shape; may be filamentous, can form branches
Reproduction	Binary fission, sometimes budding	Binary fission	Budding, fragmentation, and/or binary fission
Metabolism	Phototrophic, chemolithoautotrophic, or chemoorganoheterotrophic	Usually chemoorganoheterotrophic	Chemoorganoheterotrophic; most require cholesterol and long-chain fatty acids for growth.
Motility	Motile or nonmotile. Flagellation can be varied—polar, lophotrichous, peritrichous. Motility may also result from the use of axial filaments (spirochetes) or gliding motility.	Most often nonmotile; have peritrichous flagellation when motile	Usually nonmotile
Appendages	Can produce several types of appendages—pili and fimbriae, prosthecae, stalks	Usually lack appendages (may have spores on hyphae)	Lack appendages
Endospores	Cannot form endospores	Some groups can form endospores.	Cannot form endospores

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The Second Edition of *Bergey's Manual of Systematic Bacteriology*

- **largely phylogenetic rather than phenetic**

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Table 19.10

Organization of Bergey's Manual of Systematic Bacteriology

Taxonomic Rank	Representative Genera	Textbook Coverage
Volume 1. *The Archaea and the Deeply Branching and Phototrophic Bacteria*		
[illegible]	[illegible]	[illegible]

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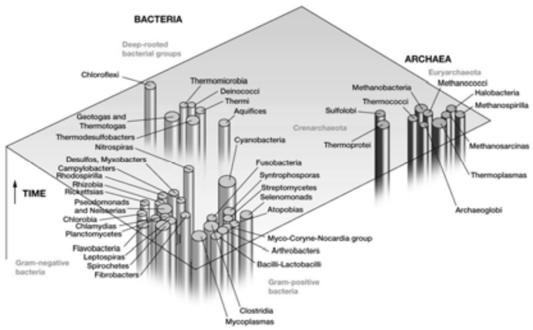

Figure 19.13

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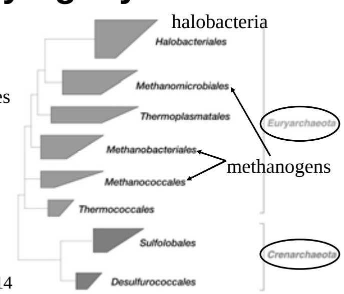
A Survey of Bacterial Phylogeny and Diversity

Archaea

two phyla
eight classes
12 orders

halobacteria

also thermophilic, sulfur reducing



many are thermophilic, sulfur metabolizing

Figure 19.14

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Domain *Bacteria*

- **metabolically and morphologically diverse**
- **divided into 23 phyla**

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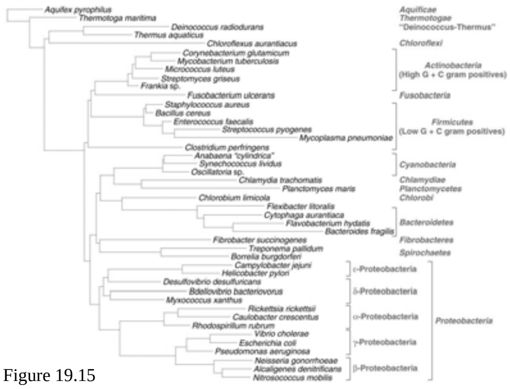

Figure 19.15

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