MOOC 4, Module 23

Classification of Bacteria (Early Approaches)

TEXT:

Although microorganisms are the smallest forms of life, collectively they constitute the bulk of biomass on Earth and carry out many necessary chemical reactions for higher organisms such as plants and animals. In the absence of microorganisms, higher life forms would never have evolved and could not now be sustained. Indeed, the very oxygen we breathe is the result of past microbial activity. Moreover, humans, plants, and animals are intimately tied to microbial activities for the recycling of key nutrients and for degrading organic matter. It is therefore quite important to say that no other life forms are as important as microorganisms for the support and maintenance of life on Earth. Therefore it is necessary to identify and group these microorganisms in different categories and hence the significance of classification lies.

The two most important terms used to study classification is “Taxonomy” and “Systematic”.

Taxonomy (Greek, taxis = arranged; nomos = law) is the classification of living organisms into groups. It deals with

- Making and maintaining collection of microorganisms
- Differentiating species
- Identification (Keys) and diagnosis of species and genera
- Naming and describing species and genera

Principles of taxonomy is an interesting tool, need to understand concept and meaning between Taxonomy, Systematic and International code of nomenclature. Taxonomy in this sense includes a range of different areas from description and naming of new taxa (nomenclature), classification and construction of identification system for particular groups of organisms.

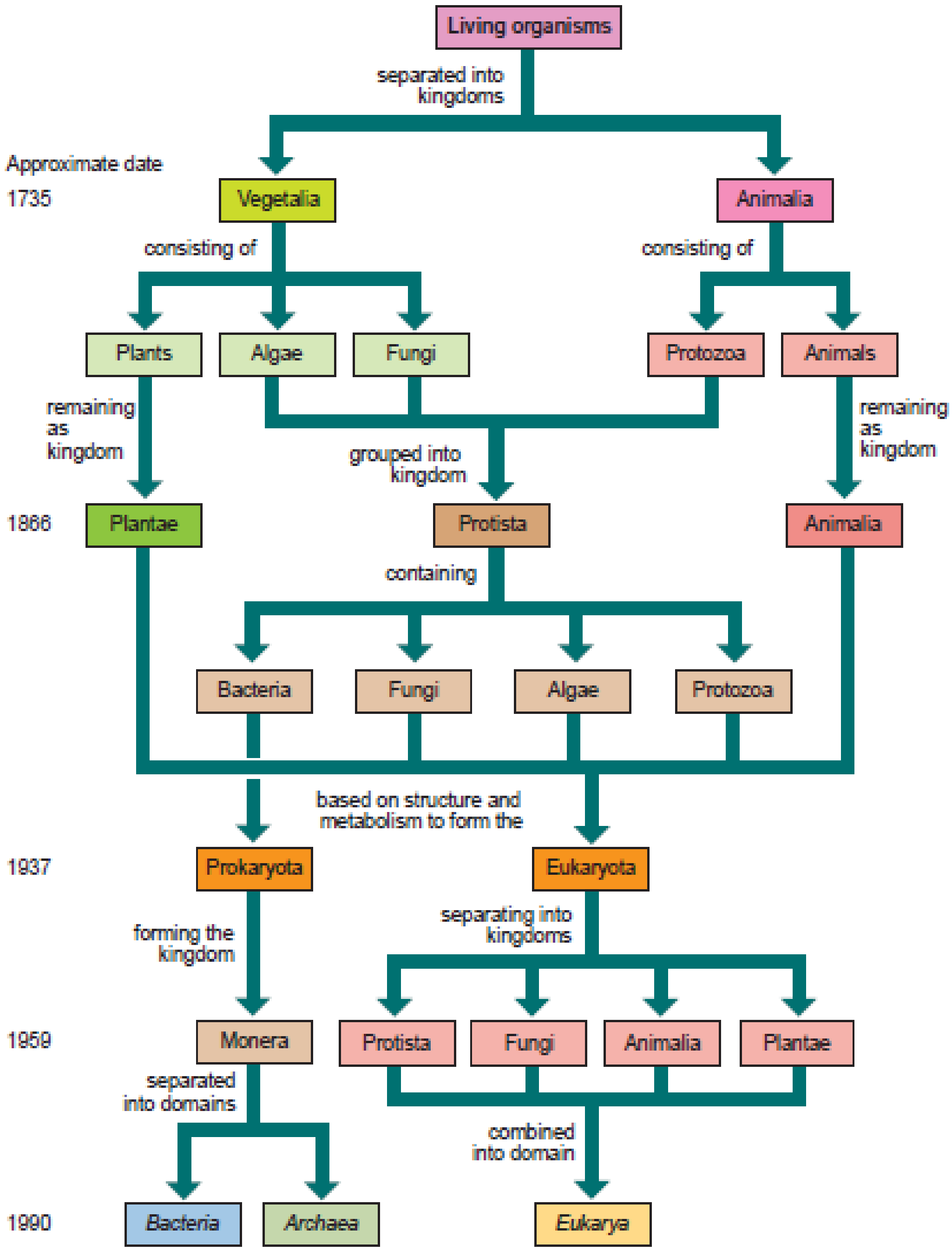
Systematic (Greek, systema = a whole made of several parts) includes traditional taxonomy with the addition of theoretical and practical aspects of evolution, genetics and speciation. The study of the evolutionary relationship between organisms is usually referred to as phylogenetics.

Systematics deals with

- Development of the classification of organisms
- Species comparison and grouping into higher categories
- Organisms are arranged in definite, hierarchical order
- The order of the system is based on hypotheses of common descent

Another goal of systematics is the naming of species and their placement in a classification. In *Systema Naturae*, Linnaeus popularized a two word (binomial) scheme of nomenclature, the two words usually derived from Latin or Greek words. Each organism’s name consists of the **genus** to which the organism belongs and a **specific epithet**, a descriptor that further describes the genus name. Together these two words make up the **species** name. For example, the common bacterium *Escherichia coli* which resides in the gut (colon) of all humans.

The term "bacteria" was traditionally applied to all microscopic, single-cell prokaryotes. However, molecular systematics showed prokaryotic life to consist of two separate domains, originally called **Eubacteria** and **Archaebacteria**, but now called *Bacteria* and *Archaea* that evolved independently from an ancient common ancestor. The archaea and eukaryotes are more closely related to each other than either is to the bacteria. These two domains, along with Eukarya, are the basis of the three-domain system, which is currently the most widely used classification system in microbiology given by Carl Woese. The following flowchart gives the development of classification system.



Development of classification system

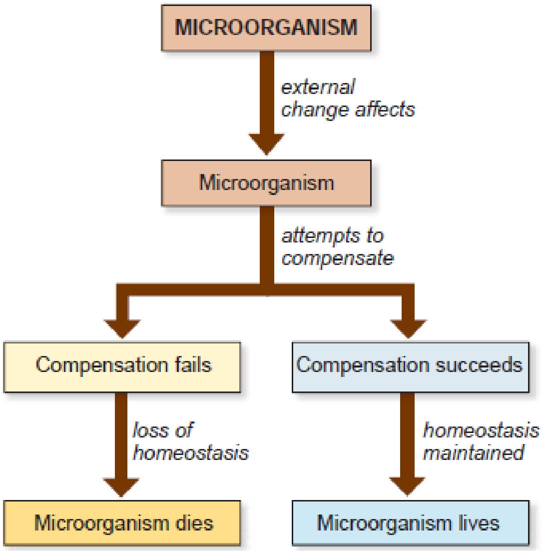
Early approaches to the bacterial Classification

Classification by morphology, biochemistry, and other features

Classification based on the morphological and biochemical features of bacteria remains the most practical way to identify the microorganisms. Between 1916 and 1918, Robert E. Buchanan wrote a number of papers on the nomenclature and classification of Bacteria, which provided the space for further research activity. It was at this time that David Hendricks Bergey, Professor of Bacteriology at the University of Pennsylvania decided to put his own studies of bacteria together with the views of other bacteriologists on classification. This led to the preparation of first edition of "**Bergey's Manual of Determinative Bacteriology"** in 1923 by the society of American Bacteriologists (now called American society of Microbiology) under the editorship of **David H. Bergey** and four others- F.C Harrison, R.S Breed, B.W Hammer and F.M Huntoon. Over the years, this authentic source of exhaustive information on bacterial classification has incorporated all significant advances as they have been made and thus has became a truly international enterprise. Since 1923, it has gone through nine editions and has generated a more comprehensive resource on classification of bacteria.

A definitive identification scheme for bacteria was first presented in 1984 in a book called **Bergey's Manual of Systematic Bacteriology**. In this scheme, bacteria are classified on the basis of many characteristics such as cell shape, cell size, colonial morphology, ultrastructural characteristics, staining behavior, cilia and flagella, mechanism of motility, shape and position of endospore in the cell, spore morphology and location, cellular inclusions, colour etc. Cell shape, motility, formation of spores and reaction to the Gram stain are important. The classification system developed at this time was the **Phenetic Classification system**. However these morphological features, including the shape and colour of bacterial colonies, are not always constant and can be influenced by environmental conditions, since the spontaneous mutation occurs in the environment. Important in the identification of a genus and species of bacteria are biochemical tests, including the determination of the kinds of nutrients a cell can use, the products of its metabolism, the response to specific chemicals, and the presence of particular characteristic enzymes. As microbiologists came to know about bacterial physiology it led to the development of **chemotaxonomy**, i.e arrangement of bacteria according to their chemical nature and metabolic/physiological activities. The chemical characters studied were cell wall constituents, carbon and nitrogen sources, energy sources, fermentation products, optimum growth temperature and range, luminescence, mechanism of energy conversion, osmotic tolerance, oxygenic relationships, pH optima and growth range, photosynthetic pigments. Salt requirements and tolerance, secondary metabolites formed sensitivity to metabolic inhibitors and antibiotics, storage inclusions etc. Other criteria used for the identification of some types of bacteria might be their antigenic composition, habitat, disease production, and requirement for specific nutrients. Some classification was designed based on the ultrastructure of the bacteria which was revealed under the electron microscope by negative staining and preparation of thin sections.

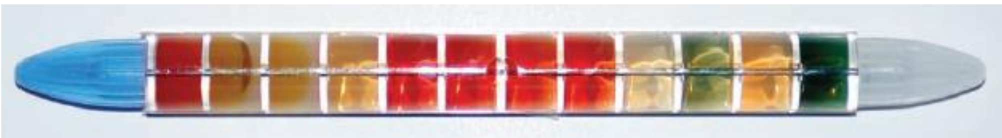
All these characters are not conserved and may change depending on the external factors that is, the compensation of the microbes to the environment. If the compensation fails, the homeostasis is lost and microorganism dies. If the compensation succeeds, homeostasis is maintained and the microbe lives. But the microbe which stays alive may not show the same morphological characters as the earlier one, while the genetic character remains same. Hence, if we follow the morphological or phenotypic characters, now the same microbe will be placed under some other genus. This was the limitation of Phenetic system.



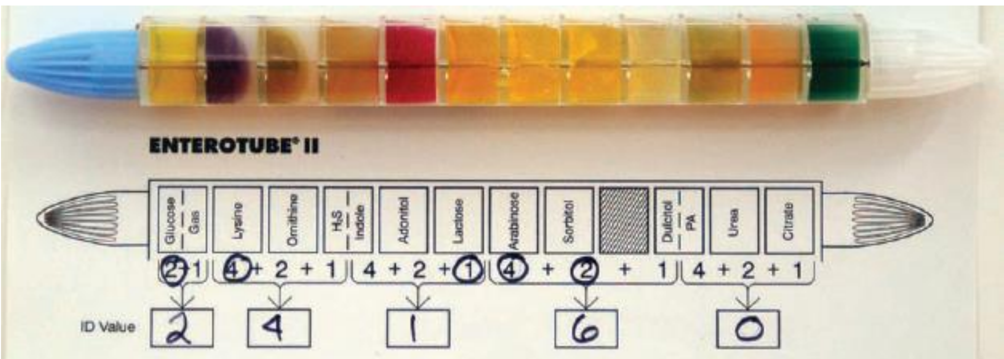
Compensation of microbes to the environment

Application of biochemical tests for identification

Enterotube II, a self-contained, sterile, compartmentalized plastic tube containing 12 different media and an enclosed inoculating wire. This system permits the inoculation of all media and the performance of 15 standard biochemical tests using a single bacterial colony. The media in the tube indicate by color change whether the organism can carry out the metabolic reaction. After 24 hours of incubation, the positive tests are circled and all the circled numbers in each boxed section are added to yield a 5-digit ID for the organism being tested. This 5-digit number is looked up in a reference book or computer software to determine the identity of the bacterium.

1. 

An uninoculated tube

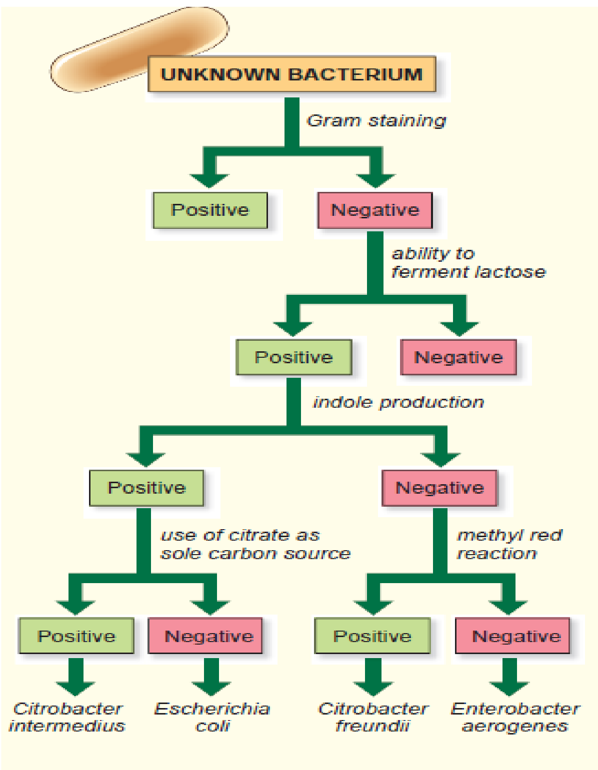
2. 

Inoculated tube after 24 hours of incubation

VALUE	IDENTIFICATION	TESTS	TESTS	
			MOT	VP
24040	*ESCHERICHIA VULNERIS	NONE	+	-
	KLEBSIELLA OZAENAE	ADO-	-	-
	HAFNIA ALVEI	ORN-	+	+
			RAF	
24060	ESCHERICHIA COLI (AD)	IND-	-	
	KLEBSIELLA OZAENAE	ADO-	+	
24061	SERRATIA LIQUIFACIENS	ORN-		
24070	ESCHERICHIA COLI (AD)	IND-		
24160	ESCHERICHIA COLI	IND-		
			PUNGENT ODOR	
24161	*SERRATIA ODORIFERA BIOGP 2	NONE	+	
24163	KLEBSIELLA PNEUMONIAE	ADO-		
24170	ESCHERICHIA COLI	IND-		
24173	KLEBSIELLA PNEUMONIAE	ADO-		
24200	KLEBSIELLA OZAENAE	ARA-		
			RAF	
24220	*SERRATIA MARCESCENS BIOGP 1	NONE	-	
	KLEBSIELLA OZAENAE	ARA-	+	
24221	SERRATIA MARCESCENS	ORN-		

3. As seen from the reference, 24160 is *Escherichia coli*.

Application of morphological and biochemical tests for identification



The morphological and biochemical characters help in identification process of four different genus.

Phenotypic classification of Bacteria

The late 19th century was the beginning of bacterial taxonomy and bacteria were classified on the basis of phenotypic markers.

- Of all the different classification systems, the **Gram stain** has withstood the test of time. Discovered by H.C. Gram in 1884 it remains an important and useful technique to this day. It allows a large proportion of clinically important bacteria to be classified as either Gram positive or negative based on their morphology and differential staining properties.
- Microorganisms can be grouped on the basis of their **need for oxygen** to grow. Facultative anaerobic bacteria can grow in high oxygen or low oxygen content and are among the more versatile bacteria. In contrast, strictly anaerobic bacteria grow only in conditions where there is minimal or no oxygen present in the environment. Bacteria such as bacteroides found in the large bowel are examples of anaerobes. Strict aerobes only grow in the presence of significant quantities of oxygen.
- Environmental reservoirs are generally divided into those that are endogenous (*i.e.*, on or within the human body) and exogenous (somewhere in the environment). When considering the likely cause of an infection the likely source of the infection is important in our differential diagnosis.

So broadly we can have three categories of bacteria namely Gram positive, Gram negative and Miscellaneous bacteria.

I. Gram Positive Bacteria

Name	Morphology	Oxygen requirement	commensals	Reservoirs/sites of colonization/ Transmission	Type of infections
Staphylococci	**Cocci in grape like cluster**	**Facultative anaerobe**	**Yes**	**Skin, nares / endogenous, direct contact, aerosol**	**Soft tissue, bone, joint, endocarditis, food poisoning**
Streptococci	**Cocci in pairs, chains**	**Facultative anaerobe**	**Some species**	**Oropharynx, skin / endogenous, direct contact, aerosol**	**Skin, pharyngitis, endocarditis, toxic shock**
Pneumococci	**Diplococci, lancet shaped**	**Facultative anaerobe**	**±**	**Oropharynx, sinus /aerosol**	**Pneumonia, otitis, sinusitis, meningitis**
Enterococci	**Cocci in pairs,chains**	**Facultative anaerobe**	**yes**	**GI tract / endogenous, direct contact**	**UTI, GI, catheterrelated infections**
Bacilli	**Rod, spore formers**	**aerobe**	**±**	**Soil, air, water, animals / aerosol, contact**	**Anthrax, food poisoning, catheter-related**

					infections
Clostridia	**Rod, spore formers**	**anaerobe**	**Some species**	**GI tract, soil / Breach of skin, endogenous, ingestion**	**Tetanus, diarrhea, gas gangrene, botulism**
Corynebacterim	**Rod, nonspore formers**	**Facultative anaerobe**	**Some species**	**Skin**	**Catheter-related infections, diphtheria**
Listeria	**Rod, nonspore formers**	**Facultative anaerobe**	**no**	**Animals, food products / Ingestion**	**Meningitis**
Actinomyces	**Irregular, filamentous**	**anaerobe**	**yes**	**GItract/endogenous**	**Skin, soft tissue**

II. **Gram Negative Bacteria**

Name	**Morphology**	**Oxygen requirement**	**commensals**	**Reservoirs/sites of colonization/ Transmission**	**Type of infections**
Enterobacteriaceae (E. coli, klebsiella, salmonella, shigella)	**Rods**	**facultative anaerobe**	**Some species**	**GI tract, animals / Endogenous, fecaloral**	**Diarrhea, urinary tract, food poisoning, sepsis**
Bacteroides	**Rods**	**anaerobe**	**yes**	**GI tract / Endogenous**	**Abscesses, intraabdominal infections**
Pseudomonas	**Rods**	**aerobe**	**No**	**Water, soil / Endogenous, breach of skin barrier**	**Infections in immunocompromised hosts, Cystic Fibrosis**
Vibrio (cholera)	**Rods, curved shape**	**Micro aerophilic**	**No**	**Water / Contaminated food, water**	**Diarrhea**
Campylobacter	**Rods, curved shape**	**Micro aerophilic**	**No**	**Food / Ingestion of contaminated food**	**Diarrhea, Bacteremia**

Legionella	**Rods, poorly stained**	**Micro aerophilic**	**No**	**Water / Inhalation of aerosol**	**Pneumonia, febrile illness**
Neisseria	**Cocci, kidneybean shaped**	**Micro aerophilic**	**No (*N.meningitidis* sometime)**	**Humans / Sexual , aerosol**	**Meningitis, pelvic inflammatory disease**
Hemophilus	**Coccobacillary - pleomorphic**	**facultative anaerobe**	**Some species**	**Respiratory tract / Endogenous, aerosol**	**Respiratory, sinusitis, otitis meningitis**
Bartonella	**Small, pleomorphic rods**	**aerobic / microaerophilic**	**No**	**Cats, fleas, lice / cat bites, lice or fleas?**	**Cat scratch disease, endocarditis, bacillary angiomatosis**

III. Miscellaneous Bacteria

Name	**Morphology**	**Oxygen requirement**	**commensals**	**Reservoirs/sites of colonization/ Transmission**	**Type of infections**
Helicobacter	**GN, but not visible on Gram stain - helical (corkscrew) shaped**	**microaerophilic**	**yes**	**Stomach / Endogenous, Fecal-oral**	**peptic ulcer disease, gastric ulcer**
Mycobacteria	**Rods, Weakly Gram positive, Acid fast stain positive**	**aerobic**	**no**	**Lungs / Fomites**	**Tuberculosis**
Treponemes	**Not visible on Gram stain, spiral shaped on dark field exam**	**noncolturable on routine media**	**no**	**Humans / Sexual transmission**	**syphilis**
Borrelia	**Not visible on Gram stain, spiral shaped on dark field exam**	**noncolturable on routine media**	**no**	**Rodents, Ticks / Tick bites**	**Lyme, Relapsing fever**
Mycoplasma	**Not visible on Gram stain, no cell wall,**	**Non-culturable on routine**	**Some species**	**Humans/ aerosol**	**Respiratory tract infections**

	pleomorphic	**media**			
Rickettsia/ Ehrlichia	**Obligate intracellular (Gram negative but not visible on Gram Stain)**	**Non-culturable on routine media**	**no**	**Ticks, Mites/ transmitted from the feces of infected lice, fleas, ticks**	**Cause a variety of illnesses including systemic vasculitis (e.g. Rocky Mountain Spotted Fever), rash, pneumonia**

Numerical Taxonomy

Numerical taxonomy improved phenotypic identification and was the first approach towards the phylogenetic relationships of prokaryotes. In this taxonomic system the properties of organism were converted into a form suitable for numerical analysis and then compared by means of computer. It includes different kinds of data: morphological, biochemical, and physiological. Many characters, at least 50 and preferably several hundred, should be compared for an accurate and reliable classification. The process begins with a determination of the presence or absence of selected characters in the group of organisms under study. 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. Sometimes the **Jaccard coefficient (**SJ**)** is calculated by ignoring any characters that both organisms lack. Here all the characteristics were given equal weightage. For example presence of capsule and similarity in genetic make-up were kept on the same platform of comparison.

Bergey's Manual of Systematic Bacteriology [First Edition 1984]

After all the information gathered with the detailed account of many microbiologists on classification, the first edition of Bergey's Manual of Systematic Bacteriology was published in the 1984. It contains both eubacteria and Archaea bacteria. The manual divides the kingdom Prokayotae into following four divisions primarily based on the nature of cell wall. These four divisions are as follows:

Division Gracilicutes

Gram-negative cell wall. Non-endospore-forming. Includes photosynthetic and nonphotosynthetic types; can exhibit swimming or gliding motility; includes rods, cocci, and curved forms.

Class Scotobacteria

Nonphotosynthetic gram-negative bacteria.

Order Spirochaetales

Spiral cells that swim by flexion; found in water and in the bodies of vertebrates; genera include *Borrelia*, *Treponema*, and *Leptospira*, all parasites of humans and other animals.

Order Pseudomonadales

Rigid-walled cells of variable shape, in some species forming chains; photosynthetic pigment present in certain species; cells usually motile by means of a single flagellum. Species found in soil, in fresh water and in salt water. Examples of genera: *Vibrio* (cholerabacteria), *Pseudomonas*, *Nitrosomonas*, *Thiobacillus*.

Order Rickettsiales

Obligate intracellular parasites; generally short rods. Multiply by binary transverse fission; often cause disease in humans and are transmitted by arthropods.

Class Anoxyphotobacteria

Gram-negative bacteria that carry out the type of photosynthesis that does not release oxygen. The major groupings within this class and some constituent genera are the purple sulfur bacteria, which use sulfide or elemental sulfur as electron donors (*Chromatium*); purple nonsulfur bacteria, which often use organic compounds as electron donors (*Rhodobacter*); green sulfur bacteria (*Chlorobium*); and filamentous green bacteria (*Chloroflexus*).

Class Oxyphotobacteria

Gram-negative bacteria that carry out oxygen-evolving photosynthesis. Includes thecyanobacteria and the order Prochlorales; gliding or nonmotile forms. Most cyanobacteria are photoautotrophs and can fix dinitrogen gas. Often form long cell filaments.

Division Firmicutes

Nonphotosynthetic gram-positive bacteria.

Class Firmibacteria

Nonbranching gram-positive bacteria. Includes rods and cocci forms. Some genera form endospores.

Class Thallobacteria

Gram-positive bacteria with branched or irregular walls. Some form spores on hyphae.

Order Actinomycetales

Rigid-walled cells that may grow out in a branching system, resembling mold colonies.Includes *Mycobacterium tuberculosis* (tuberculosis bacterium), *Streptomyces*.

Division Tenericutes

Irregular pleiomorphic cell shapes due to the absence of a rigid cell wall. Lack peptidoglycans.

Class Mollicutes

Flexible-walled cells in the order Mycoplasmatales; nonmotile, highly variable in shape at different life stages. Includes *Mycoplasma* and forms once known as pleuropneumonia-like organisms (PPLO).

Division Mendosicutes

Cell wall, when present, lacks peptidoglycan. Rods or cocci.

Class Archaebacteria

Possess cell walls and lipids with unusual compositions that differ from all other bacteria. Lipids usually are isoprenol derivatives linked to glycerol backbone through ether linkage. Ribosomes are different in protein composition and sensitivity to antibiotics than other bacterial ribosomes. Peptidoglycan, if present, does not contain muramic acid. Non-spore-forming. Most are anaerobic. None contain chlorophyll. Many are motile by flagella. Can stain gram-positive or gram-negative but have a different cell-wall appearance than do other bacteria.