

# Introduction to Prokaryotic Organisms, the Archaea and Bacteria

One of the primary references used for the identification and classification of prokaryotic organisms is the **Bergey's Manual of Systematic Bacteriology**. This reference, last published in complete form as four volumes during the mid to late -1980s, divided the prokaryotes into four major categories: the ordinary Gram-negative bacteria, the ordinary Gram-positive bacteria, bacteria with unusual properties, and bacteria with filamentous morphology. Since then, evidence gained through biochemical analysis has led to the elimination of the **kingdom Monera**, and the division of prokaryotic organisms into two distinct and separate **domains**, the **Archaea** and the **Bacteria**. A newer, and more accurate classification scheme is being incorporated into the second edition of the Bergey's Manual of Systematic Bacteriology, but the volumes addressing bacteria have not yet been published in complete form (See Appendix A in the Tortora text for a classification summary). The information included here provides an introduction to **some** of the major groups of prokaryotes as identified in the most recent editions of the Bergey's Manual available, and describes some of the ways in which these organisms are significant. Although a number of medically significant forms are mentioned in this section, they are not emphasized, and will be covered in greater detail later in the semester.

## I. The Domain Archaea:

Organisms currently categorized within the Domain **Archaea** (arch = ancient) were formerly classified as **archaeobacteria** within the Kingdom Monera; however, analyses of their nucleic acids and other molecular constituents has confirmed that the Archaea are not closely related to other prokaryotes. Microbiologists now recognize that the Archaea are unlike Bacteria in a variety of ways, and are also unlike one another. Archaea are unlike bacteria in that: 1) their **cell walls do not contain peptidoglycan**, 2) their **cell membranes contain ether-linked lipids** instead of ester-linked fatty acids, 3) their **DNA often contains introns and is associated with histone proteins** (characteristics of eukaryotic chromosomes), and 4) the **nucleotide sequences of their 5S, 16S and 23S r-RNA molecules are unlike those of other prokaryotes**. As a group the Archaea are believed to have evolved quite independently of other cell types, and often inhabit environments not formerly thought to support life. They are mostly terrestrial or aquatic, but often colonize anaerobic, hypersaline, acidic and/or hydrothermally and geothermally heated environments. According to the most recent classification scheme, the Archaea can be divided into four phyla, with the Crenarchaeota and Euryarchaeota being the most extensively studied. Although once believed to be remnants of a formerly plentiful group, the Archaea are now recognized as common organisms occupying a wide variety of unique and unexpected habitats.

**Note** – Because the Archaea were initially classified as bacteria, some names applied to the group include the term bacterium as a suffix, e.g., *Methanobacterium* and *Halobacterium*, which can cause confusion. A bacteria-like suffix or prefix is also found in the names of certain substances formed by Archaea.

Some groups of Archaea recognized as being of particular significance include the following:

## The methanogenic Archaea – Classes Methanobacteria and Methanococci:

Organisms within this group are able to produce methane as a metabolic by-product by combining hydrogen gas with carbon dioxide. They may be found in a variety of habitats including marshes, swamps, mudflats, marine sediments, and the gastrointestinal tracts of humans and other animals. Although the methane produced by these organisms is often viewed as an air pollutant, it is important as a potential energy source. Under some circumstances, the methane generated by gastrointestinal organisms (grown in vitro) is used to cook, heat buildings and generate electricity. Modern wastewater (sewage) treatment facilities require the activity of methanogenic archaea to digest organic materials in sludge. The methane produced is often captured, burned to generate heat and sometimes to run steam-driven turbines that generate electricity.

## The Extreme Halophiles – Class Halobacteria:

These unique Gram-negative organisms are able to grow only in environments having high levels of salt (17-23%). They occur naturally in salt lakes (such as the Great Salt Lake of Utah) and in association with salt production plants involving the evaporation of seawater. Members of the genus *Halobacterium* produce ATP via a unique photophosphorylation process involving a purple pigment called **bacteriorhodopsin**. Because this molecule is very similar to the rhodopsin found within the human retina, it is of great interest to scientists. It has been incorporated as a light sensitive element into artificial retinas, and specially designed optical computers.

## The Thermoacidophiles – various classes:

These organisms are unique in that they thrive in acidic, sulfur-rich hot springs where they must tolerate highly acidic conditions and high temperatures. In nature, these can grow where living conditions are unfavorable to most organisms. Some Archaea live in association with deep sea floor hydrothermal vents and tolerate extreme pressures as well as high temperatures. Recently certain Archaea have been shown to survive being autoclaved. Because they maintain their activity under extreme conditions, the enzymes produced by these archaea are of considerable interest to humans, and can be used in a variety of industrial processes.

## II. The Domain Bacteria – Not all phyla are included here:

### Phylum IV – Deinococcus-Thermus

Organisms in the genus *Deinococcus* were formerly categorized as ordinary Gram-positive cocci, but are anything but ordinary. Many species within this genus are resistant to heat, pressure, desiccation and exposure to radiation (both ultraviolet and ionizing). *Deinococcus radiodurans* is considered a **polyextremophile**, and one of the toughest types of bacteria known. They have been nicknamed "Conan the Bacterium".

The radiation resistance characteristic of *Deinococcus* is due to each cell carrying multiple copies of its genetic information and enzymes capable of repairing damaged DNA at a

rapid rate. If humans could utilize these enzymes it might be possible to develop substances to treat victims suffering from radiation poisoning, and save human lives.

Organisms in the genus *Thermus* are typically hyperthermophiles associated with hot springs and deep sea hydrothermal vents. Enzymes produced by these bacteria are capable of functioning at high temperatures and have various applications. *Thermus aquaticus*, organisms originally isolated from hot springs in Yellowstone National Park, are the source of **Taq-polymerase**, an enzyme commonly used for DNA replication in the polymerase chain reaction (PCR). You will learn more about this enzyme and its application in the laboratory.

## Phylum X – Cyanobacteria

The **Cyanobacteria** (formerly referred to as blue-green algae) are **oxygenic, phototrophic** bacteria that typically form cells much larger than those of most other bacteria. They are similar to eukaryotic algae and higher plants in that they possess true **chlorophylls** as their photosynthetic pigments, but they are not algae, because they are prokaryotic. The term **oxygenic** refers to the ability of these organisms to produce molecular oxygen (O<sub>2</sub>) in association with their photosynthetic activities (as described in detail during a later lecture). Cyanobacteria occur in a variety of shapes, and are widespread in soil, freshwater, and marine habitats. They often form symbiotic relationships with other organisms (as in lichens), and several forms produce **heterocysts**, specialized cells containing enzymes involved in nitrogen fixation, and **akinetes**, resting cells capable of surviving harsh conditions. Examples of cyanobacteria observed in the laboratory included *Oscillatoria*, *Nostoc*, *Anabaena*, *Gloeocapsa* and *Spirulina*.

Although not usually considered as pathogens, cyanobacteria have recently been implicated as agents of intoxication, i.e., substances produced by them may cause disease symptoms in humans and other animals when ingested. **Cyanotoxins** called **Microcystins** are cyclic hepta-peptide chains composed of amino acids not usually incorporated into proteins, and not formed in association with ribosomes. These peptides are highly toxic when ingested; often causing severe liver damage. Their potential toxicity in association with dietary supplements has not been fully investigated.

The cyanobacteria are not the only phototrophic bacteria, but they are the only oxygenic forms. Other bacteria categorized as **anoxygenic phototrophic bacteria** in Volume 3 of the 1989 Bergey's Manual, are now divided into several different phyla. All are Gram-negative rods, cocci, or spirilla and typically inhabit anaerobic aquatic habitats. These organisms are capable of producing ATP by means of photophosphorylation, but **do not produce oxygen as a by-product**. Four major groups within this category and specific examples within each group are: the green non-sulfur bacteria (Phylum VI Chloroflexi – genus *Chloroflexus*), the green sulfur bacteria (Phylum XI Chlorobi – genus *Chlorobium*), the purple non-sulfur bacteria (Phylum XII Proteobacteria – genus *Rhodospirillum*) and the purple sulfur bacteria (Phylum XII Proteobacteria – genus *Chromatium*). **Anoxygenic phototrophic bacteria** typically live in sediments (mud) and form **bacteriochlorophyll** pigments capable of capturing portions of the electromagnetic spectrum able to penetrate to their level. Although not always colored green or purple, they can display such colors as indicated by the color patterns visible within the Winogradsky-window displayed in the laboratory. The sulfur bacteria (green and purple) are named for their ability to

accumulate sulfur granules within their cytoplasm. These form in association with photosynthetic activities as described in a later lecture.

## Phylum XII – Proteobacteria

The phylum **Proteobacteria** is named after the mythological Greek god **Proteus**, who could assume many shapes. Organisms within this phylum form a large and diverse group with different classes designated by the Greek letters  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , and  $\epsilon$ .

Some examples in the class alphaproteobacteria include:

- 1) ***Rickettsia* and *Orientia*** - In older editions of the Bergey's manual, bacteria within the genus ***Rickettsia*** were categorized with those in the genus *Chlamydia* because both genera contained tiny, non-motile organisms nutritionally categorized as **obligate intracellular parasites** or **hypotrophs** because they are able to grow only within the cells of mammalian hosts. Organisms living exclusively within other cells often lack the cellular "machinery" (enzymes, etc.) necessary for the production of substances essential for growth and reproduction, so are dependent on their hosts. Such organisms are said to be very fastidious, and can often be cultivated only in **vivo** (i.e., inside another living organism).

The genus ***Rickettsia*** contains a number of bacteria recognized as significant human pathogens. All grow within the reticuloendothelial cells, vascular endothelial cells, or erythrocytes (RBCs) of their hosts. They are transmitted from one host to another by biting lice or ticks and enter their host's cells by inducing phagocytosis. Once inside, they reproduce rapidly. Example organisms include *Rickettsia rickettsii*, the causative agents of Rocky Mountain Spotted fever, and *R. prowazekii*, the causative agents of epidemic typhus (both spotted fevers). Organisms formerly identified as *Rickettsia tsutsugamushi* are now identified as *Orientia tsutsugamushi*, and are the causative agents of scrub typhus.

Additional pathogens related to ***Rickettsia*** include *Ehrlichia*, *Neorickettsia* and *Anaplasma* species, small, Gram-negative organisms capable of invading leukocytes, platelets and erythrocytes after being transmitted by ticks. These can cause a variety of disease symptoms in humans and other animals.

2. ***Wolbachia*** – Organisms in the genus ***Wolbachia*** are Gram-negative parasites of insects and other organisms, and estimated to be the most common reproductive parasites in the biosphere. Within their arthropod hosts, ***Wolbachia*** species commonly infect the ovaries and/or testes, bringing about reproductive changes variously resulting in:
  - a) **death** of infected males,
  - b) **feminization**, i.e., causing males to develop as females or sterile pseudomales,
  - c) **parthenogenesis**, i.e., causing females to reproduce successfully without males (the result being an all female population),
  - d) cytoplasmic incompatibility, i.e., leaving infected individuals unable to mate with others infected by different ***Wolbachia*** strains.

Several different arthropod species are so influenced by ***Wolbachia*** infection that they are unable to reproduce if not carrying the bacteria inside their bodies.

*Wolbachia* species may have significant impact on human health worldwide because they can also infect a variety of **filarial nematodes** known to parasitize humans; e.g., *Dirofilaria immitis* (canine heartworms), *Onchocerca volvulus* (nematodes responsible for causing river blindness), and *Wuchereria bancrofti* (nematodes responsible for causing elephantiasis). The disease symptoms associated with filarial nematode infection appear to be due largely to the immune system's response to *Wolbachia*; furthermore, eliminating *Wolbachia* from the nematode parasites (currently accomplished with antibiotics), typically results in nematode death or sterility, potentially reducing disease symptoms. Controlling *Wolbachia* might also provide a means for reducing the prevalence of insects serving as vectors for viral, bacterial and protozoan pathogens.

Since global climate change is likely to increase the range of the mosquitoes and biting flies responsible for transmitting filarial nematodes and other pathogens, recognition of *Wolbachia* species and their role as **symbionts** may be critical to maintaining human health.

3. *Rhizobium* – Organisms in the genus *Rhizobium* are important nitrogen "fixers" recognized for their ability to develop complex symbiotic relationships with the roots of plants sometimes called legumes (e.g., beans, peas, alfalfa, soybeans, clovers and lupines). The *Rhizobium* cause the formation of root nodules, and then join with plant cells to form **leghemoglobin**, a red-colored, oxygen-absorbing pigment. This reduces the free-oxygen concentration within the nodule, potentially increasing the efficiency of nitrogen-fixing enzymes, and may also transfer oxygen to the respiratory bacteria. Bacteria living within root nodules tend to increase the nitrogen content of soils where legumes are grown, thus reducing the need for commercial fertilizers. Though farmers have long recognized the benefits of crop rotation, i.e., planting alfalfa, clover or soybeans as a cover crop to improve soil fertility, they often give credit to the wrong organisms. The nitrogen "fixers" are bacteria, not plants.

The possibility of establishing symbiotic relationships between *Rhizobium* species and plants used as food crops, e.g., wheat, rice, corn and other grains, has prompted extensive research in this area; however, the ultimate solution may involve genetically modifying plant species to make them capable of fixing nitrogen on their own. Either scenario could increase agricultural yields from poor quality soils, and help feed the dangerously dense human population currently inhabiting the planet earth.

4. *Agrobacterium* – Bacteria in the genus *Agrobacterium* are close relatives to *Rhizobium* and recognized as plant pathogens capable of transferring genes between their cells and those of their host plants. *Agrobacterium tumefaciens* is responsible for causing **crown gall disease**, an ailment characterized by the formation of tumor-like growths (galls) at the junction between plant roots and stems. Crown gall formation occurs after genes are transferred from tumor-inducing plasmids within the *Agrobacterium* cells. The ability of *Agrobacterium* species to transfer genes into plants has prompted their use in biotechnology; specifically, modifying their plasmids to allow for the transfer of genes into plants without causing disease. Several different agricultural varieties have been genetically modified using modified *Agrobacterium tumefaciens* plasmids as vectors for gene transfer.

*Azotobacter*, another genus containing nitrogen-fixing bacteria and one subject of the soil enrichment exercise conducted in the laboratory, is categorized within the class gammaproteobacteria. Multiple other bacteria genera can fix nitrogen.

Additional pathogens categorized within the class alphaproteobacteria include *Bartonella* and *Brucella*, genera recognized as animal pathogens sometimes transmitted to humans by arthropod vectors or by other means.

Some examples in the class betaproteobacteria include:

1. *Sphaerotilus* – Bacteria in the genus *Sphaerotilus* were classified as bacteria with unusual properties in the 1989 version of the Bergey's Manual because they are **sheathed bacteria**. Sheathed bacteria are Gram-negative, aerobic rods that form and reside within tube-like structures called **sheaths**. Bacterial sheaths are typically organic in composition, and usually thin and colorless. In water containing iron, they may appear yellow-brown in color due to encrustation with ferric iron, and resemble rust-colored, underwater spider webs.

*Sphaerotilus natans* is a common inhabitant of water environments including slow-moving freshwater streams, wastewater treatment facilities, wells, and fishtank filters. The sheaths generated by these tiny bacilli sometimes cause significant disruption of water flow, potentially problematic to humans; but the bacteria do not pose a health hazard.

2. *Thiobacillus* – Bacteria in the genus *Thiobacillus* were categorized as **chemolithotrophic bacteria** in the 1989 edition of the Bergey's Manual. They are chemoautotrophs important in maintaining environmental mineral cycles called **biogeochemical cycles**. Members of the genus *Thiobacillus* are common soil inhabitants recognized as "sulfur metabolizing bacteria" because they convert sulfur-containing compounds such as elemental sulfur, sulfides, and sulfites, into sulfates. Plants can then incorporate these sulfates into amino acids necessary for building proteins. Bacteria identified as *Thiobacillus ferrooxidans* are acidophiles used in some commercial mining operations because they can oxidize iron and inorganic sulfur compounds. These organisms use hydrogen sulfide as an energy source and release sulfuric acid as a byproduct. The acid can dissolve minerals such as copper and uranium from low-grade ores making the recovery of these materials cost effective. *Thiobacillus* can also be used to remove sulfur from coal, thereby reducing the potential for air pollution when coal is burned.

*Nitrosomonas* is another genus associated with biogeochemical cycles, but these are involved in the nitrogen cycle. They convert ammonia to nitrite, which can then be converted to nitrate by members of the genus *Nitrobacter*. These organisms, sometimes called "nitrifying bacteria" play an essential role along with nitrogen "fixers" in maintaining soil fertility.

3. *Neisseria* – Bacteria in the genus *Neisseria* include two species pathogenic to humans; *Neisseria gonorrhoeae*, the causative agents of gonorrhoea, and *Neisseria meningitidis*, causative agents of meningitis.

Some examples in the class gammaproteobacteria include:

1. *Pseudomonas* – Members of the genus *Pseudomonas*, sometimes referred to as pseudomonads, are Gram-negative bacilli common in soil and water environments; some species infect plants or animals. Recall that *Pseudomonas*, along with *Azotobacter* were among the subjects of the soil enrichment exercise we conducted in the laboratory. Nearly all pseudomonads are motile by polar flagella, and in culture tend to be "swarmers". Many produce a variety of non-photosynthetic pigments, and have characteristic odors. Two species commonly utilized in our laboratory are *Pseudomonas aeruginosa*, important human pathogens, and *Pseudomonas fluorescens*, common soil and water inhabitants capable of producing a yellow-green, water-soluble pigment, fluorescent when exposed to ultraviolet light.

Several *Pseudomonas* species are unique in their ability to utilize a remarkably diverse assortment of organic compounds as carbon and energy sources. This allows them to utilize a variety of pollutants inadvertently released into the environment by human activities, e.g., insecticides, herbicides, brake fluid, motor oil, gasoline, and materials worn from automobile tires running incessantly on roadways. The pseudomonads thus serve, along with many other bacteria, as microbes engaged in environmental cleanup. Humans, having recently gained an appreciation for such activity, now employ *Pseudomonas* species along with other microorganisms in a process called **bioremediation**. Populations of microbes maintained in specific locations or carried to specific sites will consume toxic chemical pollutants, thus eliminating the necessity of storing them, or illegally dumping them into the environment (a practice all too common among industries). *Pseudomonas* species are generally among the microbes utilized to clean up oil spills following "mishaps" involving petroleum transport.

*Pseudomonas syringae* is a species known to produce cell surface protein molecules that promote the formation of ice crystals. These organisms are often used as nucleating centers in large scale "snow" making operations associated with ski resorts. A genetically modified strain of *Pseudomonas syringae*, designated as "ice-minus", lacks the ability to produce the surface proteins, and when applied to vegetation, helps prevent the formation of ice crystals potentially damaging to crops. The use of these genetically modified bacteria to protect food crops from frost damage remains somewhat controversial, but could save farmers millions of dollars in damage each year.

2. *Photobacterium* – Members of the genus *Photobacterium* live in marine environments where they often form symbiotic relationships with bioluminescent organisms such as squids and various types of deepwater fish. **Bioluminescent** organisms have the ability to emit light, but in the case of squids and fish, light production is actually attributed to *Photobacterium phosphoreum* living on their surfaces. These bacteria produce pigments called **luciferins** (Lucifer = light bringer) that serve as substrates for **luciferase enzymes**. Oxidation of luciferins by luciferase enzymes results in the release of energy in the form of blue-green light. Flashlight fish, lantern fish, and other organisms support patches of *Photobacterium* in regions called "light organs", and can often "turn" these lights off and on by covering and uncovering the bacteria. The fish use the light to attract prey species or potential mates, and the bacteria are provided with a living space and food supply (a nice example of mutualism). The

*Photobacterium* species use **quorum sensing** to regulate their light production and only emit light when their populations reach a specific density.

**Luciferins** and **Luciferase enzymes** are encoded by regions of DNA called **lux genes**, and are found within a variety of other bioluminescent organisms including fireflies. Lux genes have multiple applications in biotechnology, e.g. restriction mapping and tracing the movement of pathogens within host organisms.

Rumor has it that by genetically modifying tobacco plants with lux genes, researchers were able to create a tobacco product that would "light by it-self".

Bacteria identified as *Vibrio fischeri* are also bioluminescent and sometimes associated with marine organisms. Other members of the genus *Vibrio* are important human pathogens, e.g., *Vibrio cholerae* (the causative agents of cholera) and *Vibrio parahaemolyticus* (organisms associated with both skin and gastrointestinal infections).

### 3. *Escherichia coli* and "friends" – (the Enteric or Coliform bacteria)

Gram-negative bacteria identified as *Escherichia coli* are one species among many within the family Enterobacteriaceae. These organisms are all closely related to one another, and can be identified only through biochemical and/or serological testing. Several species are considered to be part of the "normal flora" found within the large intestine or colon of humans and other animals, and so are referred to as **coliforms**. Additional important genera within the family include *Salmonella*, *Shigella*, *Enterobacter*, *Serratia*, *Proteus*, *Klebsiella*, *Citrobacter* and *Yersinia*. Several of these will be encountered as participants in our physiological unknown set, but others, including *Shigella* and *Yersinia* are pathogens not welcome in our laboratory (*Yersinia pestis* are recognized as the causative agents of bubonic plague).

Discovered by Theodore Escherich in 1885, populations of *Escherichia coli* have been maintained in microbiology laboratories since, and are among the most thoroughly studied organisms on the planet. Some strains, e.g., O:157-H:7, are respected as serious pathogens responsible for food recalls and some human deaths, but others live as normal flora within our guts and are far more beneficial than harmful. Your *E. coli* strains produce **vitamin K**, and **colicins**, chemicals inhibiting the growth of more virulent *E. coli* strains. Some important human applications of *Escherichia coli* include:

- a) water testing, i.e., using *E. coli* as indicators of fecal contamination in water,
- b) research, i.e., *E. coli* strains have served as common subjects in genetic research,
- c) recombinant protein production, i.e., *E. coli* strains can be genetically modified and grown commercially to produce proteins initially found in other organisms, including humans. Proteins including human insulin (humulin), human growth hormone (Nutropin or Humatrope) and Factor VIII (Kogenate) can be produced by *E. coli*.

Some other genera in the class gammaproteobacteria are recognized as containing potential human pathogens; these include *Legionella*, *Haemophilus*, *Coxiella*, *Moraxella*, *Acinetobacter*, *Aeromonas*, and *Pasteurella*. Organisms in the genus *Legionella* are usually free-living in water or damp soils, but are opportunistic pathogens of humans that can cause legionellosis or **Legionnaire's Disease**. These bacteria often colonize water supply lines and can grow and reproduce within fresh-water amoebae, making



them difficult to eradicate. *Haemophilus influenzae* (formerly called *Bacillus influenzae*) were named for their apparent association with the influenza epidemic of 1918. These organisms do not cause influenza, but are potential pathogens responsible for causing epiglottitis and meningitis in young children (disease is prevented by application of the Hib vaccine). The genome of *Haemophilus influenzae* was one of the first ever to be sequenced.

Some examples in the class Deltaproteobacteria include:

1. *Bdellovibrio* – Bacteria in the genus *Bdellovibrio*, are unique in that they attack and kill other Gram-negative bacteria (bdella = leech and vibrio = comma-shape). Initially thought to be intracellular parasites, *Bdellovibrio* cells actually feed on host polymers (proteins and nucleic acids) from within the periplasmic space, and do not actually enter the cytoplasm. To gain access to a host cell a *Bdellovibrio* bacterium collides with the outer membrane of the cell wall at speeds up to 160µm/second using a single, polar flagellum as propulsion. After attaching to the host, the *Bdellovibrio* "drills" its way inside, seals the hole formed in the membrane, and then converts the cell into a **spheroplast** called a bdelloplast. Within the periplasm, a *Bdellovibrio* cell may remain dormant for some time, not adversely affecting its host; but eventually **hydrolase enzymes** are used to digest host cell molecules, making nutrient rich monomers available for *Bdellovibrio* metabolism and growth. The *Bdellovibrio* elongates, forming a filament that will eventually break into multiple, new, flagellated cells (up to 80 within long, flagellated *E. coli* hosts). When released, these bacterial parasites are free to attack new cells.

Other bacteria known to attack and kill microorganisms are classified within the genera *Bacteriovorax* and *Vampirovibrio* (parasites of unicellular algae called *Chlorella*).

2. **Desulfo-bacteria** – Organisms referred to as **Desulfo-bacteria** in the 1989 edition of the Bergey's manual are categorized within numerous genera all beginning with the prefix **Desulfo**; including, *Desulfovibrio*, *Desulfococcus*, *Desulfospira*, *Desulfobacter*, etc. (more than 25 genera). All of these organisms are capable of reducing elemental sulfur and sulfur-containing compounds, resulting in the formation of hydrogen sulfide (H<sub>2</sub>S) gas. Hydrogen sulfide gives rotten eggs their characteristic foul odor, and is one of several metabolic by-products causing bacterial cultures and environments supporting them to "stink". Desulfo-bacteria play essential roles in biogeochemical cycles involving sulfur, and provide **anoxygenic photoautotrophs** with a source of electrons essential to ATP synthesis (as described in a later lecture).

Some examples in the class epsilonproteobacteria include *Helicobacter pylori* (the causative agents of gastric ulcers) and *Campylobacter jejuni* (gastrointestinal pathogens of humans and other animals).

### Phylum XIII – Firmicutes

Bacteria in the phylum Firmicutes were categorized as ordinary Gram-positive bacteria in the 1986 edition of the Bergey's Manual. Some important genera in this phylum include:

1. *Clostridium* – Bacteria within the genus *Clostridium* are primarily anaerobic, endospore-forming bacilli, typically associated with soil and/or water. In most species

the development of an endospore causes the sporangium to be swollen or distended. Several species including *Clostridium tetani*, *Clostridium botulinum*, and *Clostridium perfringens* are important human pathogens known to form potent exotoxins encoded by viral genes. Common gut inhabitants identified as *Clostridium difficile* can cause serious diarrhea and potentially death in patients taking antimicrobial drugs because eliminating normal flora allows virulent strains of these organisms to overgrow and produce damaging levels of toxins.

2. *Mycoplasma* – Bacteria in the genus *Mycoplasma* appear as tiny Gram-negative bacteria when observed in Gram stain preparations, and were formerly categorized as such. Nucleic acid studies indicate they are more closely related to Gram-positive bacteria in the phylum Firmicutes (the low G + C % Gram-positive bacteria). These organisms are highly **pleomorphic** (variable in shape) because they lack a peptidoglycan cell wall and sometimes form filaments resembling fungi, hence the name myco (mykes = fungi). Due to their tiny size (0.1 to 0.25µm in diameter), they often pass through bacterial filters and were initially thought to be viruses. They are now believed to represent the smallest self-replicating life forms. Their lack of a rigid cell wall allows them to be flexible and so variable in shape, but also causes them to be quite susceptible to changes in osmotic pressure. One type of bacteria within this group, *Mycoplasma pneumoniae* is a significant human pathogen.
3. *Bacillus* - Bacteria in the genus *Bacillus* are common aerobic and facultatively anaerobic, endospore-forming bacilli found in association with soil and rotting vegetation. Some are thermophilic, some produce antibiotics, and some are pathogenic to humans and/or other animals. The genus *Bacillus* has recently been divided, and some species moved to related genera including *Paenibacillus*, *Lysinibacillus* and *Brevibacillus*. Some *Bacillus* and Bacillus-like organisms of particular interest include:
  - a) *Bacillus thuringiensis* – *Bacillus thuringiensis* are significant insect pathogens whose endospores and crystalline protein by-products are used as biological insecticides (sometimes sold as BT or Dipel). These materials will kill the larvae of butterflies and moths feeding on crop plants without harming humans. About 170 naturally occurring Bt toxins are known to exist, and genes encoding certain forms have been placed into genetically modified crop plants. Although this has significantly reduced the need for applying insecticides to crops, it has also been damaging to populations of certain butterfly species not considered as "pests".

Organisms identified as *Lysinibacillus sphaericus* can also serve as biological insecticides, but kill mosquitoes rather than butterflies and moths. Bacteria identified as *Paenibacillus* larvae also kill insects and are responsible for causing a disease of honeybees called American foulbrood. Currently, an increase in antibiotic resistance among these bacteria is causing serious damage to commercially maintained honeybee populations, and financial difficulties for both bee keepers and the farmers whose crops the honeybees usually pollinate. This may ultimately result in increased food prices for consumers.

- b) *Bacillus anthracis* – *Bacillus anthracis* are recognized as pathogens capable of causing anthrax in humans and other animals. Recent findings indicate that *Bacillus anthracis*, *Bacillus thurengiensis* and *Bacillus cereus* (a common inhabitant of

our laboratory) are all the same organism type; with differences between them being encoded by genes carried on plasmids.

- c) *Bacillus subtilis* – Bacteria identified as *Bacillus subtilis* are the source of the antibiotic Bacitracin (a non-ribosomal peptide). Bacteria identified as *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) produce antibiotics called polymyxins.
4. *Staphylococcus* – Bacteria in the genus *Staphylococcus* appear as Gram-positive cocci typically arranged in grape-like clusters. They are catalase-positive and in some cases form yellow-colored carotenoid pigments within their colonies. Various *Staphylococcus* species are found in association with humans and other animals, typically living on the skin and in nasal passages. Bacteria identified as *Staphylococcus aureus* are important pathogens, recognized as the causative agents of a variety of human disorders, and are often associated with **noscomial** (institutionally acquired) infections. Cultures of *Staphylococcus aureus* and *Staphylococcus epidermidis* are used in our microbiology laboratory.
5. *Lactobacillus* - Organisms in the genus *Lactobacillus* are slender, Gram-positive bacilli not capable of forming endospores. Most are free-living forms and some are of considerable interest to humans. Sometimes referred to as lactic acid bacteria because they form lactic acid as their primary fermentation product, different *Lactobacillus* species are used extensively in the production of cheese, yogurt, sauerkraut and sourdough bread. The unique flavor of San Francisco style sourdough bread is attributed to *Lactobacillus sanfranciscensis* (formerly *Lactobacillus sanfrancisco*).
6. *Lactococcus* – Organisms in the genus *Lactococcus* were formerly categorized within the genus *Streptococcus*, but are now considered to represent a unique species. *Lactococcus lactis* (subspecies *lactis* and *cremoris*) are organisms commonly used in the production of cheese.
7. *Streptococcus* – Organisms in the genus *Streptococcus* are typically non-motile (or rarely motile), catalase-negative, homofermentative short bacilli (cocci when grown in broth media), arranged in pairs or short chains. Most streptococci are parasites of humans and other animals, and several forms are pathogenic. Two clinically significant forms are *Streptococcus pyogenes* (causative agents of strep throat, scarlet fever and rheumatic fever) and *Streptococcus pneumoniae* (the most common cause of bacterial pneumonia).

Bacteria identified as *Streptococcus thermophilus* are lactic acid bacteria important in the processing of various cultured foods including cheese and yogurt. The lactic acid and acetaldehyde produced as fermentation products by these organisms add a distinctive flavor to these foods.

## Phylum XIV – Actinobacteria

Many organisms currently incorporated into the phylum **Actinobacteria** were previously categorized with other organisms since shown to be unrelated. Some examples of bacteria in this phylum include:

1. *Micrococcus* – Bacteria in the genus *Micrococcus* are non-motile, oxidative, catalase-positive, Gram-positive cocci often occurring as inhabitants of soil, water and the skin surfaces of humans and other animals. The genus formerly included a large number of species characterized by their ability to form distinctly colored colonies in pink, orange, yellow, or white when grown on nutrient agar. Most of these are now divided among other genera including *Arthrobacter*, *Kocuria*, *Kytococcus* and *Dermacoccus*. Species from these groups commonly form colonies on nutrient agar plates exposed to air, and are often incorporated into our morphological unknown set because they are easily grown in vitro, are rarely pathogenic, and are readily visible in stained preparations.
2. *Corynebacterium* – Bacteria in the genus *Corynebacterium* are Gram-positive, irregularly shaped bacilli, sometimes with enlarged or club-shaped ends. They often contain **metachromatic granules** (also called volutin granules) and form pallisading or "snapping" aggregations in stained preparations.

*Corynebacterium diphtheriae* are recognized as the causative agents of diphtheria, an important disease affecting only humans. Disease symptoms are caused by a protein exotoxin encoded by viral genes, but not all strains are toxigenic.

3. *Mycobacterium* – Bacteria in the genus *Mycobacterium* are obligately aerobic bacilli common in soil and water environments. They have Gram-positive type cell walls, but are resistant to staining due to the high levels of **mycolic acid** (a wax-like lipid) present. Once stained, these organisms resist decolorizing, even when exposed to acid-alcohol, so are **acid-fast**. Most *Mycobacterium* species grow slowly when cultivated and some can be grown only on living surfaces. Many species are free-living saprotrophs, while others are important human pathogens. *Mycobacterium leprae*, the causative agents of leprosy (Hansen's disease) and *Mycobacterium tuberculosis*, the causative agents of tuberculosis are of particular concern.
4. *Nocardia* – Bacteria in the genus *Nocardia* are catalase-positive, aerobic, Gram-positive organisms that tend to form mats of branching, thread-like strands (hyphae) on the surface of agar media. Individual rod-shaped, Y-shaped or coccoid cells are released from these mats via fragmentation. Some *Nocardia* species are partially acid-fast and some have been identified as opportunistic pathogens.
5. *Streptomyces* – Bacteria in the genus *Streptomyces* are Gram-positive, filamentous organisms commonly found in soil and rotting vegetation. Most species form asexual reproductive bodies called **conidia** or **conidiospores** at the tips of branching filaments, causing colonies growing on agar surfaces to have a powdery surface texture.

Members of the genus *Streptomyces* are a primary source for new antimicrobial drugs and are currently recognized as the source for a large number of antibiotics including Tetracycline, Streptomycin, Neomycin, Erythromycin, Vancomycin and Chloramphenicol. Different species are also the source of an anti-cancer drug called Migrastatin, and antifungal drugs including Amphotericin B.

Colonies of *Streptomyces* growing on agar plates are said to emit a characteristic "earthy" odor. This is because *Streptomyces* species typically produce a volatile metabolite called **geosmin**. Since geosmin is responsible for the pleasant odor of freshly tilled earth, or rain-soaked soil, dirt actually smells like *Streptomyces*.

Cyanobacteria living in soil also produce geosmin, which is often concentrated in the tissues of root vegetables such as beets and fish living in water bodies supporting large populations of cyanobacteria. Geosmin can cause foods to taste "muddy" and therefore unpalatable, but fortunately cooking with lemon juice or vinegar will break down the geosmin, improving the flavor.

## Phylum XVI – Chlamydiae

The most commonly recognized members of the phylum Chlamydiae are categorized within the genus *Chlamydia*, and are recognized as important human pathogens.

1. *Chlamydia* – Bacteria in the genus *Chlamydia* are Gram-negative, but unlike other bacteria, lack peptidoglycan in their cell walls. They reproduce only inside host cells where they typically undergo unique developmental cycles involving variations in cell types, i.e., forming elementary, intermediate and reticulate bodies. Although they are **hypotrophs**, the *Chlamydia* do not require vectors for transmission. They are transmitted to humans through interpersonal contact or may become airborne and infect via the respiratory tract. Once thought to be unable to produce ATP, *Chlamydia* cells are now known to have limited ATP synthesis ability. *Chlamydia trachomatis* are the most important, and are recognized as human pathogen causing both trachoma, an infection of the eye often resulting in blindness, and a sexually transmitted urethritis similar to gonorrhea. *Chlamydia* cause the most commonly reported sexually transmitted disease in the United States, and according to the CDC were estimated to infect more than two billion people during 2006.

## Phylum XVII – Spirochaetes

Bacteria in the phylum Spirochaetes, commonly called **spirochetes**, are Gram-negative organisms with flexible cell walls and axial filaments or periplasmic flagella, i.e., flagella located within their periplasmic space. They are generally very thin, and so poorly visible with normal light microscopy.

Some spirochetes are free-living aquatic forms, others are parasitic, and some are pathogenic to humans. Three genera of particular importance to humans are *Treponema* (the causative agents of syphilis), *Borrelia* (the causative agents of relapsing fever and Lyme disease), and *Leptospira* (the causative agents of systemic infections resulting in fever, jaundice, and meningitis).