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*. The first edition of the *Bergey's Manual of Determinative Bacteriology* was published in 1923 in association with what is now the American Society for Microbiology (ASM). Nine editions of this manual were published (the last in 1994) and a four-volume set called the *Bergey's Manual of Systematic Bacteriology* was published in 1984-89. This reference, currently available in our laboratory, 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 was incorporated into the second edition of the *Bergey's Manual of Systematic Bacteriology* (published in 2001-2012, and also available in the laboratory), but even this was soon outdated as new organisms were isolated and characterized (about 100 new genera and 600 plus new species per year between 2010 and 2015). Most recently (since April of 2015) an on-line version called the *Bergey's Manual of Systematics of Archaea and Bacteria* (BMSAB) has been made available. This provides the most complete and authoritative description of bacterial and archaeal diversity, and includes taxonomy, systematics, ecology, physiology and other biological properties of all described prokaryotic taxa.

This section provides an introduction to some of the major groups of prokaryotes included in the most recent editions of the Bergey's Manual, and describes some of the ways in which these organisms are significant. Although a number of medically important 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. Some of the metabolic pathways used by Archaea are more similar to those used by eukaryotic organisms, as are some features of Archaeal DNA. Microbiologists now recognize that the Archaea are unlike Bacteria in a variety of ways, and are also unlike one another. Some of the ways Archaea differ are listed below.

1. The cell walls of Archaea are unlike those of bacteria in that they do not contain peptidoglycan. Peptidoglycan (sometimes called murein) contains polysaccharides connected together by short peptide chains, and although Archaea form a wall material called pseudomurein (pseudopeptidoglycan), it lacks polysaccharides and contains multiple layers of protein arranged in a complex array called an S-layer. Some types of Archaea (e.g., *Thermoplasma* and *Ferroplasma*) lack cell walls.

2. Archaeal cell membranes are unique, i.e., unlike those found in other cell types. Archaeal membranes contain ether-linked lipids instead of ester-linked fatty acids, and these are attached to mirror image glycerol molecules (ether linkages are chemically stronger bonds).
Some Archaeal membranes are lipid monolayers as the isoprenoid (side-branching) lipids extend between two glycerol molecules.

3. Archaeal DNA molecules are typically circular (as they are in most bacteria) and often contain introns and are associated with histone proteins (like eukaryotic chromosomes). The transcription (RNA synthesis) and translation (protein synthesis) processes in Archaea are more similar to those found in eukaryotic cells than to those used by bacteria.

4. The nucleotide sequences of their 5S, 16S and 23S rRNA molecules are unlike those of other prokaryotes. The 16S rRNA gene nucleotide sequences were initially used by Carl Woese to separate the Archaea from Bacteria (establish two separate domains).

5. Archaea have multiple unique enzymes, coenzymes and metabolic processes, i.e., unlike those used by other cell types. For example, the enzymes used to form ether-linked lipids and the enzymes used to form methane are not found in other types of cells. Bacteriorhodopsin and halorhodopsin, proteins used for capturing light energy (photophosphorylation) are made only by archaea.

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 multiple phyla, with the Crenarchaeota and Euryarchaeota being the most extensively studied. Additional phyla include Nanoarchaeota (a phylum with only one representative), Micrarchaeota and Parvarchaeota (some of the smallest life forms known), Thaumarchaeota, Aigarchaeota and Korarchaeota (sometimes grouped together in a superphylum with Crenarchaeota) and the more recently proposed phyla Lokiarchaeota, Thorarchaeota, Odinarchaeota and Heimdallarchaeota (grouped together in the superphylum Asgard, named for the realm of gods in Norse mythology). Representatives from most of these new phyla are being identified in association with habitat sampling (because they cannot be grown under typical laboratory conditions) and their classification remains somewhat controversial. Although once believed to be remnants of a formerly plentiful group, the Archaea are now recognized as common organisms occupying a wide variety of habitats including soils, oceans and marshlands. Like bacteria, archaea form part of the human microbiota, inhabiting the gastrointestinal tract, mouth and skin surfaces. There are still some archaea found only in unique and unexpected habitats, but certainly not all of them. No archaea are believed to be pathogenic.

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, e.g., bacteriorhodopsin.

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

The methanogenic Archaea – Classes Methanobacteria and Methanococci:

Organisms categorized as methanogenic archaea are Euryarchaeota able to produce methane as a metabolic by-product. Some, e.g., Methanobacterium, form methane by combining hydrogen gas with carbon dioxide (a process requiring the use of unique coenzymes such as coenzyme M and methanofuran), while others form methane and carbon dioxide through the catabolism or organic compounds (e.g., alcohols, acetic acid and formic acid).
Methanogenic archaea can be found in a variety of habitats including marshes, swamps, mudflats, marine sediments, and the gastrointestinal tracts of humans and other animals. None appear to be pathogens, but several form symbiotic relationships (typically mutualistic) with other organisms. Archaea living in the gastrointestinal tracts of termites and ruminants appear to assist the cellulose-digesting protozoa present. Cellulose catabolism results in the release of hydrogen and carbon dioxide, but high levels of hydrogen can interfere with ATP production. By converting hydrogen and carbon dioxide into methane, the methanogenic archaea help the protozoa form ATP. Methanogenic archaea identified as *Methanobrevibacter smithii* are common in the human gut and are thought to aid digestion by interacting with other gut organisms.

Although the methane produced by methanogenic archaea is often viewed as an air pollutant, it is important as a potential energy source for human use. 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:**

Extreme halophiles are unique Gram-negative Archaea 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/pink pigment called *bacteriorhodopsin* (described in detail later). 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; most representatives described are culturable organisms and numbers are from the textbook (TFC, Microbiology, 12th Ed.)*

**Phylum II – 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, dessication 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.

**Phyla III, V and VI – Anoxygenic phototrophic bacteria**

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 III - Chloroflexi – genus *Chloroflexus*), the green sulfur bacteria (Phylum V - Chlorobi – genus *Chlorobium*), the purple non-sulfur bacteria (Phylum VI - Proteobacteria – genus *Rhodospirillum*) and the purple sulfur bacteria (Phylum VI - 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 photophosphorylation activities as described in a later lecture.

Organisms identified as *Rhodospirillum rubrum* grow readily, though slowly, under aerobic conditions at room temperature (within lab drawers). These organisms can produce bacteriochlorophylls and carotenoid pigments within pink-colored colonies even when grown in the dark. Without light availability, photophosphorylation cannot occur and the cells use the organic compounds in the medium rather than light as their source of energy, i.e., they become chemoheterotrophs rather than photoheterotrophs.

**Phylum IV – 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 (recall, cyanobacteria functioning as endosymbionts gave rise to chloroplasts). The term *oxygenic* refers to the ability of these organisms to produce molecular oxygen (O₂) in association with their photophosphorylation 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 (e.g., in lichens and within the water fern *Azolla*), and several forms produce *heterocysts*, specialized cells containing enzymes involved in nitrogen fixation,
and akenetes, 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 heptapeptide chains composed of amino acids not usually incorporated into proteins, and not formed in association with ribosomes. These peptides (released when cells die) are highly toxic when ingested; often causing severe liver damage. Their potential toxicity in association with dietary supplements has not been fully investigated, but regular consumption of Spirulina may pose a health hazard as these organisms have been found to form microcystins.

Phylum VI – 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 α, β, γ, δ, and ε.

Class Alphaproteobacteria – Some examples 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 hypotrophs (obligate intracellular parasites) 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 tumifaciens* 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 tumifaciens* plasmids as vectors for gene transfer.
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.

**Class Betaproteobacteria** – Some examples 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.

   Bacteria identified as *Sphaerotilus natans* are common inhabitants of water environments including slow-moving freshwater streams, wastewater treatment facilities, wells, and fish tank filters. The sheaths generated by these tiny bacilli sometimes cause significant disruption of water flow, potentially problematic to humans; but the bacteria do not otherwise 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.

3. *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* (Alphaproteobacteria). These organisms, sometimes called "nitrifying bacteria" play an essential role along with nitrogen "fixers" in maintaining soil fertility.

4. *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.

**Class Gammaproteobacteria** – Some examples 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 may become "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 (tanker or pipeline failures).

*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. *Azotobacter* – Bacteria in the genus *Azotobacter* form larger cells than most other Gamma-proteobacteria (2µm in diameter) and mucoid colonies when grown on enrichment medium. Members of the genus *Azotobacter* are **nitrogen-fixing** bacteria commonly associated with soil and the surfaces of plant leaves. Like *Pseudomonas*, these were the subject of the soil enrichment exercise conducted in the laboratory. Many other bacteria genera contain species that can fix nitrogen.

3. *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* (or other species) 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). These *Photobacterium* species use **quorum sensing** to regulate their light production and only emit light when their populations reach a specific density.
4. **Vibrio** - Bacteria in the genus *Vibrio* are comma-shaped organisms commonly associated with seawater or brackish water found in estuaries. *Vibrio fischeri* are bioluminescent and like *Photobacterium* are sometimes associated with marine organisms. Other members of the genus *Vibrio* are important human pathogens, e.g., *Vibrio cholerae* (the causative agents of cholera), *Vibrio parahaemolyticus* (organisms associated with both skin and gastrointestinal infections), and *Vibrio vulnificus* (an opportunistic pathogen capable of infecting individuals when open wounds are exposed to marine or estuarine environments).

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

Gram-negative bacteria identified as *Escherichia coli* are one species among many within the Order *Enterobacteriales* and sometimes referred to as enterics. These organisms are all closely related to one another, and often identified through biochemical and/or serological testing. Several species are considered to be part of the "normal microbiota" found within the large intestine or colon of humans and other animals, and so are referred to as *coliforms*. Additional important genera within the order 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 (organisms identified as *Yersinia pestis* are recognized as the causative agents of bubonic plague).

Discovered by Theodore Escherich in 1885, populations of *Escherichia coli* (E. coli) have been maintained in microbiology laboratories since, and are among the most thoroughly studied organisms on the planet. Some strains, e.g., the serotype O:157:H:7, are respected as serious pathogens responsible for food recalls and some human deaths, but others live as normal microbiota 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 epiglotitis and meningitis in young children (disease is
The genome of *Haemophilus influenzae* was one of the first ever to be sequenced.

**Class Delta-proteobacteria** – Some examples 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 160mm/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*, *Desulfospiria*, *Desulfo bacter*, 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 (H₂S) 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).

Bacteria identified as *Desulfovibrio desulfuricans* are anaerobic, sulfate-reducing organisms found in marine sediments, sewage and soils. They have been found to produce **methylmercury**, a powerful neurotoxin in humans. The formation of methylmercury is significant because it tends to accumulate within animal tissues and negatively impact ecosystem food webs.

**Class Epsilon-proteobacteria** - Some examples include:

1. *Helicobacter* – Bacteria identified as *Helicobacter pilori* are the causative agents of gastric ulcers. These bacteria are microaerophils that use chemotaxis to direct their movement through the mucus lining the stomach to reach the less acidic epithelial cells beneath (they sometimes enter these cells). Most people carrying *Helicobacter pilori* in their stomachs never experience symptoms or complications.

2. *Campylobacter* – Bacteria identified as *Campylobacter jejuni* are gastrointestinal pathogens of humans and other animals. Typically acquired through ingestion, these can cause dysentery, fever and pain, sometimes accompanied by vomiting 24-72 hours after infection.
Phylum VII – 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 other members of the normal microbiota allows virulent strains of these organisms to overgrow and produce damaging levels of toxins.

2. **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 species moved to new genera including *Paenibacillus, Lysinibacillus, Brevibacillus* and several others. 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 beekeepers 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).

   d) **Paenibacillus polymyxa** – Bacteria identified as *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) produce antibiotics called polymyxins.
3. **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 carotinoid 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 nosocomial (institutionally acquired) infections. Cultures of *Staphylococcus aureus* and *Staphylococcus epidermidis* are used in our microbiology laboratory and multiple other species have been isolated by students. Bacteria identified as *Staphylococcus lugdunensis* (part of our normal microbiota) produce an antibiotic called Lugdunin that inhibits the growth of *Staphylococcus aureus*.

4. **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 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*).

5. **Lactococcus** – Organisms in the genus *Lactococcus* were formerly categorized within the genus *Streptococcus*, but are now considered to represent a unique group. *Lactococcus lactis* (subspecies *lactis* and *cremoris*) are organisms commonly used in the production of cheese.

6. **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 VIII – Tenericutes**

1. **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) than to any Gram-negative forms. 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 0.25mm in diameter), they often pass through bacterial filters and were initially thought to be viruses. They are now believed to represent one of 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 causing “walking” pneumonia.
2. *Erysipelothrix* – Bacteria identified as *Erysipelothrix rhusiopathiae* are Gram-positive, catalase-negative bacilli distributed worldwide in soil and water contaminated by infected animals. These organisms cause a disease known as erysipelas in various animals, but most commonly in pigs, turkeys and laying hens. Infection in pigs typically results in skin lesions, arthritis, endocarditis and sepsis; it is of economic importance to the pig industry.

**Phylum IX – 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, Kyctococcus* 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.

   *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.

   *Corynebacterium pseudotuberculosis* causes an infection in horses and other animals called pigeon fever (due to swollen abscesses resembling a pigeon’s breast). These bacteria live in soil and are transmitted to horses through mechanical abrasion or insect bite. External abscesses drained onto soil will spread the organisms.

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.

   *Mycobacterium leprae*, the causative agents of leprosy (Hansen’s disease) and *Mycobacterium tuberculosis*, the causative agents of tuberculosis are of particular concern.

3. *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 were initially recognized as the source for a large number of antibiotics including Tetracycline, Streptomycin, Neomycin, Erythromycin, Vancomycin and Chloramphenicol (changes in taxonomy have led to name changes for some of these). 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.

6. *Bifidobacterium* – Bacteria in the genus *Bifidobacterium* are Gram-positive, non-motile, often branching bacilli that inhabit primarily anaerobic environments in the mouth, vagina and gastrointestinal tract. *Bifidobacterium infantis* (a biotype of *B. longum*) is typically one of the first types of bacteria to colonize the human gut, and may represent up to 90% of the infant gut microbiome. These organisms are now known to play a crucial role in infant development. They carry enzymes allowing them to break down the human milk oligosaccharides (HMOs) abundant in breast milk and to produce a variety of substances beneficial to infants. They form short chain fatty acids that nourish intestinal cells while inhibiting the growth of yeast and other fungi, they also form sialic acid necessary for normal brain development, and folic acid (vitamin B9) necessary for RBC development, DNA synthesis and growth. *Bifidobacterium infantis* also crowd out unwanted Gram-negative cells, signal the infant gut to produce proteins that reduce gut permeability, and help stimulate normal immune responses.

*Bifidobacterium longum* subspecies *infantis* 35624 is marketed as a **probiotic** and sold under the brand name Align in the US and Canada, and as Alflorex in Ireland, the UK and other European countries. These *Bifidobacterium* are also associated with other prescription preparations used to treat ulcerative colitis.

**Phylum XI – 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 most 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 XII – 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).

**Phylum XIII – Bacteriodetes**

Bacteria in the phylum Bacteriodetes are Gram-negative, non-sporing, aerobic or anaerobic bacilli common in soil, water and in the guts and on the skin of animals. Our morphological unknown identified as *Sphingobacterium composti* is a member of this group.

**Phylum XIV – Fusobacterium**

Bacteria in the genus *Fusobacterium* are anaerobic, Gram-negative, non-sporing bacilli with pointed ends. Though previously considered part of our normal microbiota, evidence suggests these organisms are human pathogens. In the mouth they are associated with periodontal disease, they are associated with Lemierre’s syndrome (thrombophlebitis of the internal jugular vein) and skin lesions. Evidence suggests they may assist other pathogens by helping to anchor biofilms.