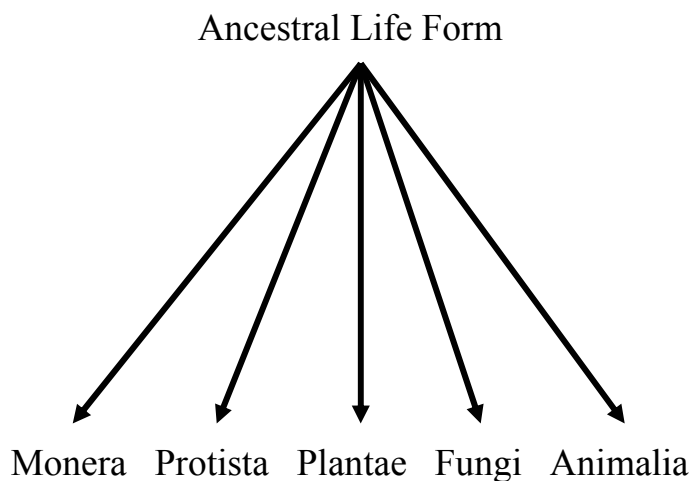


## Taxonomy

The term taxonomy alone is enough to cause glazing over of students' eyes in traditional Biology courses. The significance of taxonomy cannot be overlooked as it is the basis for categorizing organisms. It is very important for the student to determine the factor(s) or characteristic(s) of the organism which cause the specific life form to be included and excluded by various taxa or categories.

Years ago organisms were classified as plants or animals. The first scientist known for his work in this arena was Carl von Linne. He categorized 11,000 organisms with genus and species epithets. As more information became available it was clear that these categories did not cover all life-forms. Whittaker's 5-kingdom system was subsequently accepted. In this system organisms were categorized in five major divisions as follows:



Increasing technological proficiency has enabled scientists to establish new relationships between organisms based on similarities in biochemistry. The most widely accepted method of classification is the Three Domain System.

Domain Prokarya (bacteria, blue-green algae)

Domain Archaea (non-nucleated cells found in extreme environments)

Domain Eukarya

Kingdoms:

Diplomonadida (diplomonads, two nuclei, no mitochondrion)

Parabasala (Trichomonads, no mitochondria)

Euglenozoa (Euglena)

Alveolata (dinoflagellates, apicomplexans, amoeba, forams, actinopoda, slime molds)

Stramenopila (diatoms, brown alga, water molds)

Rhodophyta (red algae)

Chlorophyta (green algae)

Mycetozoa (slime molds)

Fungi (molds, cup fungi, mushroom)

Plantae (mosses, ferns, evergreens, flowering plants)

Animalia (sponges, invertebrates, vertebrates)

## **Domain Bacteria Laboratory**

### **Objectives:**

1. Describe the main difference between Domain Archaea and Domain Eubacteria.
2. Describe the main characteristics of methanogens, extreme thermophiles and halophiles.
3. Describe general characteristics of domain Eubacteria (domain Bacteria).
4. Describe general characteristics of Cyanobacteria and their role in ecology.
5. Demonstrate the occurrence of bacteria in nature by culturing microbes from multiple sources.
6. Describe the appearance and function of bacterial endospore.
7. Describe the economic significance of bacteria.

### **Prokaryotes**

The former kingdom Monera is divided into two Domains under currently accepted taxonomic classification systems. The first Domain is that of Bacteria. It is further subdivided into about a dozen bacterial groups, five of which are:

1. Spirochetes
2. Chlamydias
3. Gram-positive bacteria
4. Cyanobacteria, and
5. Proteobacteria.

The second domain is the Domain Archaea, which includes prokaryotes, which inhabit the most extreme and harsh environments of the planet:

1. Methanogens
2. extreme halophiles, and
3. extreme thermophiles.

Prokaryotes are virtually ubiquitous!! They are everywhere! Both in respect to numbers and impact, they dominate the biosphere. Most prokaryotes are very small. Often they are 1/10-1/100 of the size of a eukaryotic cell. It is estimated that the earliest of prokaryotes developed life forms about 3.5 billion years ago. Some of them function as decomposers. Life is possible for all as they assist in recycling inorganic compounds required by plants.

Emphasis is placed on anti-microbial disinfectants and cleaners today but only a small number of bacteria are pathogenic (disease-causing.) Many prokaryotes live in close associations with each other as well as eukaryotes in what are called symbiotic relationships. Lyn Margulis proposed The *Theory of Endosymbiosis*. She suggested that some organelles of the eukaryote (chloroplasts and mitochondria) existed originally as independent single-celled organisms, which were prokaryotes.

While a few thousand prokaryotes are known, it is estimated that perhaps millions exist in nature. There are many diverse forms capable of unusual metabolic pathways. It is within these simplest of life forms that more complex biochemical pathways originated.

The structure of prokaryotes is rather simple when compared with cells of higher organisms. The cell wall, constructed largely of peptidoglycan, gives structure to the cell. It is very different from the cell wall of various eukaryotes. Classification placed them with plants in the antiquated 2-kingdom system. External to this cell wall are other materials that constitute a type of extracellular material, which does differ from species to species.

One of the most well used methods of classification involves use of the Gram stain. Bacteria can be classified as Gram-positive if they stain purple via the technique whereas Gram-negative bacteria remain pink and do not capture the Crystal Violet-Iodine complex due to less peptidoglycan in the wall. Gram-negative bacteria possess an outer membrane, which consists of lipopolysaccharides (carbohydrates and lipids.) One of the mechanisms by which prokaryotes are often limited by antibiotics is by prevention of the cross-linkage in the cell wall.

Another layer, the capsule, is formed by many prokaryotes. It is a sticky substance that forms yet another protective layer. Capsules also allow the bacteria to adhere to substrata as well as to each other in colonial form. Sometimes surface appendages called pili promote adherence of these cells

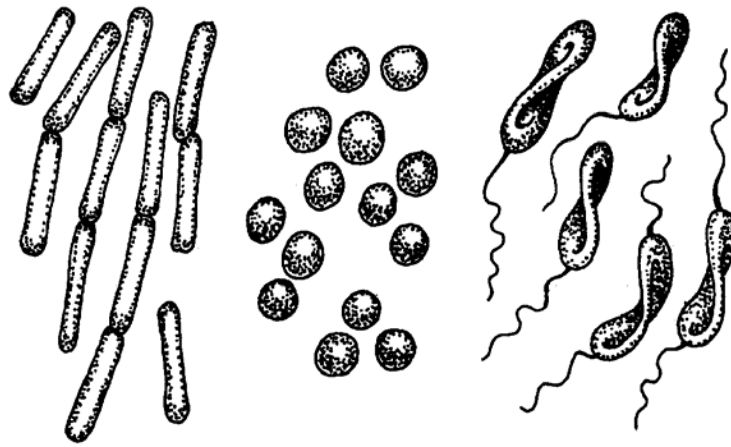
to mucous membranes or to each other in exchanging plasmids (small circular loops of DNA that confer advantages such as resistance to antibiotics.)

While no organelles exist as in eukaryotic cells, many of the biochemical pathways still exist in association with infoldings of membrane (mesosomes.) The DNA tends to be naked and occurs in a snarl of fibers, the nucleoid. Ribosomes exist and function in a similar manner to those in eukaryotes; however, they are smaller and less complex. Taxis, movement toward or away from a stimulus, occurs in response to food, light, magnetic forces or gravity. Some cells possess a single flagellum or flagella, occasionally under the outer membrane, as in spirochetes.

Cyanobacteria can exist under very harsh conditions and formerly were classified with plants not only because of the cell wall but also because many contained various pigments and were able to carry out photosynthesis with the net production of oxygen. Some of the earliest fossils of life forms are stromatolites, layers of cyanobacteria that became fossilized. Some cyanobacteria are able to carry out nitrogen fixation whereby atmospheric  $N_2$  is converted to  $NH_3$ , which is then used to synthesize amino acids. The gelatinous capsule and toxins make them a poor food source for predators. While these algae are often referred to as the blue-green algae, they are not always that color. Many possess chlorophyll a, as in plants, but also accessory pigments that change the absorption spectrum for the specific plant.

Prokaryotes may appear in colonies, in bunches or strings of cells. Monerans that we will look at today include various forms of bacteria. Some of these may be on prepared slides. Note that observed internal cell structure is not discernible with the techniques that you will be using today. Cell walls and capsules will be seen with stains. Remember that bacteria are very simple life forms but are ubiquitous and extremely important. Bacteria play a major role in decomposition of organic material, in disease as well as antibiotic production.

Typical form is in the spherical shape, coccus, rod-shaped bacillus or spiral shaped spirochete. It is very important to use the oil immersion technique when examining them microscopically.

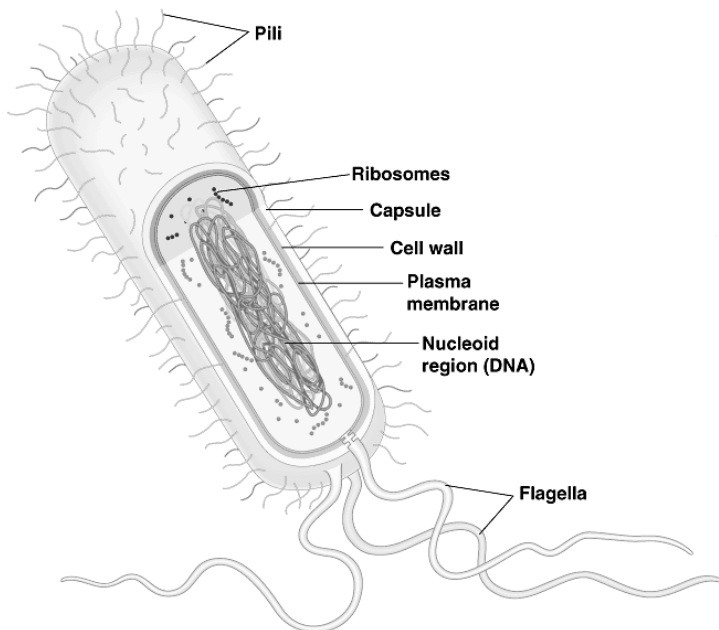


bacilli

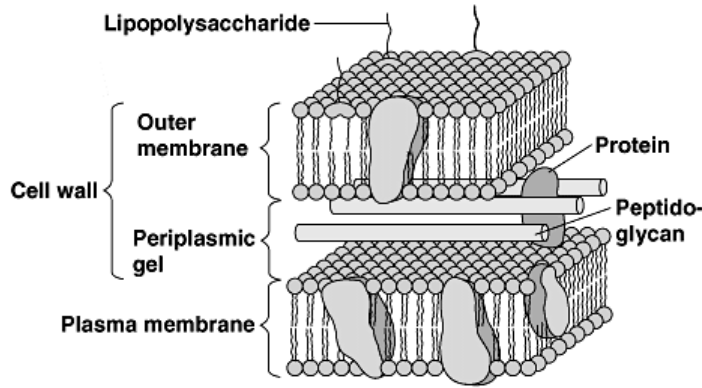
cocci

spirilla

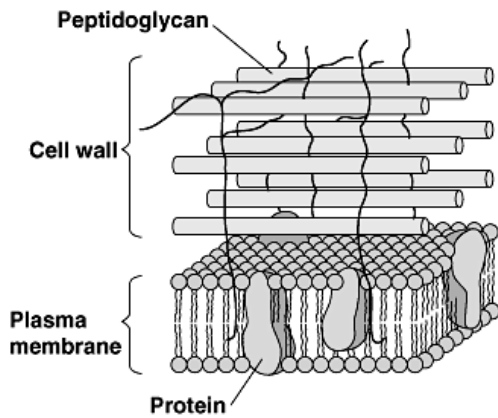
Structures present include cell wall, capsules outside wall, pili, endospores, flagella (simple), nucleoid, plasmids, ribosomes and mesosomes (infoldings of the plasma membrane associated with enzymes for specialized reactions like respiration).



Gram positive bacteria possess more peptidoglycan (polymers of sugars and amino acids) in a simpler cell wall while gram negative bacteria have a more complex wall with less peptidoglycan.

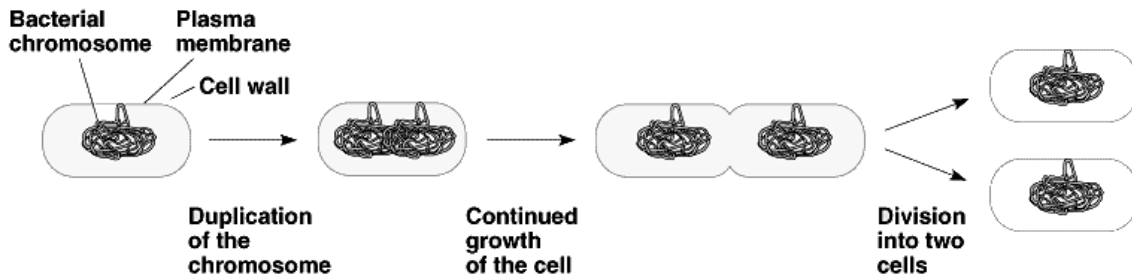


Gram negative membrane



Gram positive membrane

Growth occurs by a process called binary fission. The DNA is replicated and attached to the plasma membrane. The membrane slowly pinches off as the cell wall and capsule are added in small increments. Replication can occur as often as every 20 minutes with sufficient elimination of waste products and availability of nutrients.



Endospores are tough capsules that form around the nucleoid yielding a structure that is resistant to extreme conditions. Only certain bacteria form endospores but these must be exposed to extreme conditions to destroy the endospore. Endospores can survive in honey which is generally a great

bacteriacidal agent. It is not recommended that infants be fed honey for this reason. *Clostridium botulinus* which is responsible for food poisoning can be fatal.

Genetic recombination occurs in a number of ways. Transformation involves taking up genes from the environment. Conjugation involves transferring genes from bacterium to bacterium. The mode of transduction allows for genes to be transferred to bacteria via viruses.

All known nutritional modes evolved in prokaryotic cells. The table below lists the four modes prokaryotes can be divided into using energy source (phototroph versus chemotroph) and carbon source (autotroph versus heterotroph) as the criteria.

	<u>Nutritional Mode</u>	<u>Energy source</u>	<u>C source</u>
1.	photoautotrophs	light	CO <sub>2</sub>
2.	chemoautotrophs	inorganic chemicals	CO <sub>2</sub>
3.	photoheterotrophs	light	organic compounds
4.	chemoheterotrophs	organic chemicals	organic compounds

Relationships with other organisms include bacteria as saprobes and symbionts (mutualism, commensalism, parasitism.) Nitrogen fixation is significant as prokaryotes are responsible for using atmospheric nitrogen and converting it to ammonia for use by plants. Denitrifying bacteria carry out the opposite process.

Obligate aerobes include those bacteria that require oxygen as an electron receiver in cellular respiration. Facultative anaerobes can operate with oxygen present or harvest energy through fermentation, which of course does not harvest as much energy as would be provided through oxidative phosphorylation. Obligate anaerobes are often poisoned by oxygen and participate in cellular respiration and electron transport with another molecule acting as an electron acceptor at the end of the ETC (Electron Transfer Chain).



The first prokaryotes (3.5 billion years ago) were probably chemoheterotrophs. ATP might have been the first nutrient. Evolution of glycolysis probably occurred in a step by step process. The origin of ETC was probably early in the energy harvesting process. Proton pumps originally were used to expel extra protons to the environment. Ultimately the cells were able to pair the flow of protons into cell with the phosphorylation of ADP via chemiosmosis.

Photosynthesis developed a bit later. The original function of pigments was probably as a shield from light. Bacteriorhodopsin, a pigment, used light to pump protons out of some cells. Later, other photosystems developed that could generate reducing power in the form of NADPH by driving electrons from hydrogen sulfide to NADP<sup>+</sup>.

The evolution of specialized bacteria (cyanobacteria), capable of oxygenic photosynthesis, began the oxygen revolution. Oxygen was released and the atmosphere became more oxidizing. This resulted in great changes in the ecosystems.

Many eubacteria are responsible disease in humans; about 50% of all human disease is caused by bacteria. Our immune system is generally able to protect us from these pathogenic bacteria. Periodically, however, the bacterium evades the body's defenses and illness occurs. Other pathogens are opportunistic. These bacteria are normal residents of the human body but only cause illness when the body's defenses are weakened.

Robert Koch, a German doctor, was the first to make the connection between a disease and the specific bacterium that caused it. Koch established four criteria, now called Koch's postulates, for establishing a pathogen as the specific cause of a disease. These guidelines are still applied to associate microbes and resulting diseases today. It is necessary to find the same pathogen in each case of the disease, isolate and culture the microbe, reinfect a population and isolate and culture it once again.

Some pathogenic bacteria cause illness by disrupting the health of the host. However, most bacteria cause illness by producing poisons called exotoxins and endotoxins.

Exotoxins are typically soluble proteins secreted by living bacteria during exponential growth. The production of the toxin is generally specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin while nonvirulent strains do not, and the toxin is the major determinant of virulence (e.g. tetanus and diphtheria). At one time it was thought that exotoxin production was limited mainly to Gram-positive bacteria, but both Gram-positive and Gram-negative bacteria produce soluble protein toxins. Bacterial protein toxins are the most powerful human poisons known and retain high activity at very high dilutions. Endotoxins are part of the outer membrane of the cell wall of Gram-negative bacteria.

Endotoxins are invariably associated with Gram-negative bacteria whether the organisms are pathogens or not. The term "endotoxin" is occasionally used to refer to any cell-associated bacterial toxin. However, it more properly refers to lipopolysaccharide toxins associated with the outer membrane of some Gram-negative bacteria (for example, *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, and *Haemophilus*).

### **Using the Oil Immersion Objective**

Filling the space between the slide and the lens with oil increases the resolution of the image. Oil has a much higher index of refraction than air and contributes to the magnification of the image. The following directions must be followed carefully to avoid damage to the microscope or to the slides.

1. Locate the object and center it with scanning lens.
2. Move to low, and then to high power, refocus while using the fine adjustment knob only.
3. Swing the high power lens away from the slide. Place a drop of immersion oil on the center of the coverslip and move the oil immersion lens into position. Refocus using the fine adjustment knob only.
4. When you are finished, swing the lens away. Do not move any other lens into position.
5. Remove the slide and wipe most of the oil off with paper, then clean with soap and water.
6. Clean the lens with xylene and then dry lens paper.

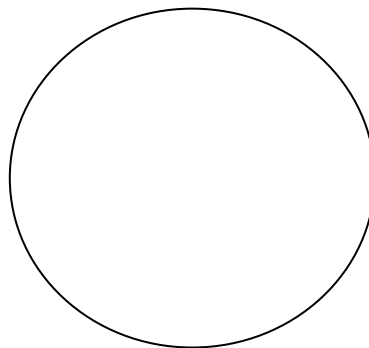
7. Students MUST show the microscope to professor before placing in the cabinet.

The bacteria that you will examine are found in yogurt with live cultures of bacteria. The bacteria commonly used are *Streptococcus thermophilus*, which ferments the sugar lactose, and *Lactobacillus bulgaricus*, which produces the flavors and aroma of yogurt.

**Prepare a slide of yogurt culture:**

1. Obtain a slide and cover slip. With a bacterial loop or a toothpick transfer a small amount of yogurt to the center of the slide.
2. Smear the yogurt in an area slightly smaller than the cover slip. Allow it to dry.
3. Place two drops of crystal violet or carbolfuchsin stain on the air-dried yogurt smear.
4. Place a coverslip on the stained smear and examine under the microscope.

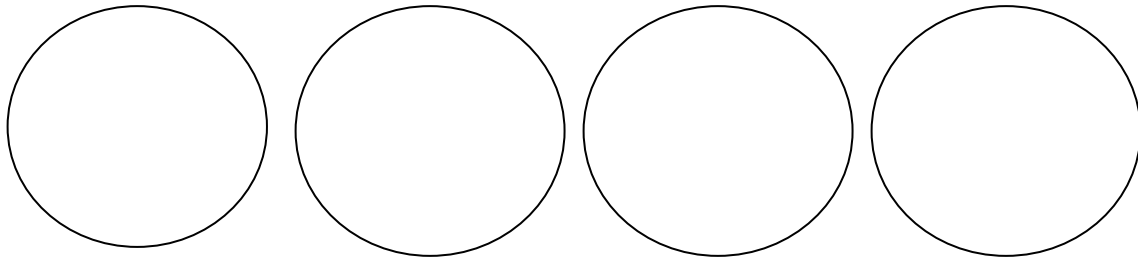
Draw the observed bacterial types in the space below.



Cyanobacteria are blue-green algae. Blue-green algae is found in aquatic environments as well as in damp, terrestrial environments. They exist largely as colonies and filaments and produce spores that resist desiccation. They generally have gelatinous capsules that are often toxic and are not a normal food source for heterotrophs.

You may be using prepared slides or using fresh cultures of these organisms. A few cyanobacteria are Anabeana, Gloeocapsa, Oscillatoria and Merismopedia. Your instructor may provide a survey culture of various organisms to key out and to exam.

Draw and label several representatives of cyanobacteria in the space below.



Anabeana

Gloeocapsa

Merismopedia

Oscillatoria

Often the source of a specific disease or type of contamination can be determined by culturing microbes, growing them under rather specific conditions (media, temperature, and gasses) and using indicators to demonstrate specific reactions that are being carried out.

Your instructor may give you nutrient agar plates. The instructor will give directions as to the manner in which they shall be used. Students will use sterile swabs to inoculate the Petri dishes. Various surfaces may be swabbed to check for presence of various types of prokaryotes. Definitive techniques will not be used at this level to attempt to identify specific genera. Plates should be sealed with tape and placed in a safe place for 2-3 days. Each round colony that grows is likely to be the result of a single bacterium's growth.

### **Laboratory Questions:**

1. Differentiate between Bacteria and Archaea.

2. Describe the morphology of bacteria.

3. Compare and contrast nutritional modes of bacteria.

4. Discuss Koch's postulates.

5. Compare and contrast modes of genetic recombination in bacteria.

## Strategies for Obtaining Energy and Carbon

	Source of Carbon	
Energy Source	Autotrophs: synthesize reduced organic molecules from CO <sub>2</sub> , CH <sub>4</sub> , etc	Heterotrophs: use reduced organic molecules from other organisms
Light (phototrophs)	Photoautotrophs: Cyanobacteria use photosynthesis as source of ATP; fix CO <sub>2</sub> in Calvin cycle	Photoheterotrophs: use photosynthesis as source of ATP; absorb reduced carbon molecules from environment (eg Heliobacteria)
Reduced organic molecules (organotrophs)	<i>Clostridium acetivum</i> ferments glucose to produce ATP; fixes CO <sub>2</sub> using acetyl-CoA pathway	Chemoorganotrophs: <i>E. coli</i> uses fermentation or respiration of glucose to form ATP; absorbs reduced carbon molecules from environment
Reduced inorganic molecules (lithotrophs)	Chemolithotrophs: <i>Nitrosomas</i> produces ATP via respiration using NH <sub>3</sub> as electron acceptor; fixes CO <sub>2</sub> via Calvin cycle	Chemolithotrophic heterotrophs: <i>Beggiatoa</i> produces ATP via respiration using H <sub>2</sub> S as electron donor; absorbs reduced carbon molecules from environment

### Some electron donors and acceptors used by Bacteria and Archaea

Electron donor	Electron acceptor	Product	Category
H <sub>2</sub> or organic molecules	SO <sub>4</sub> <sup>2-</sup>	H <sub>2</sub> S	Sulfate-reducers
H <sub>2</sub>	CO <sub>2</sub>	CH <sub>4</sub>	Methanogens
CH <sub>4</sub>	O <sub>2</sub>	CO <sub>2</sub>	Methanotrophs
S or H <sub>2</sub> S	O <sub>2</sub>	SO <sub>4</sub> <sup>2-</sup>	Sulfur bacteria
Organic molecules	Fe <sup>3+</sup>	Fe <sup>2+</sup>	Iron-reducers
NH <sub>3</sub>	O <sub>2</sub>	NO <sub>2</sub> <sup>-</sup>	Nitrifiers
Organic molecules	NO <sub>3</sub> <sup>-</sup>	N <sub>2</sub> O, NO or N <sub>2</sub>	Denitrifiers (nitrate reducers)
NO <sub>2</sub> <sup>-</sup>	O <sub>2</sub>	NO <sub>3</sub> <sup>-</sup>	Nitrosifiers

### Some Diseases Caused by Bacteria

Bacterium	Lineage	Tissues affected	Disease
<i>Chlamydia trachomatis</i>	Planctomyces	Urogenital tract	Genital tract infection
<i>Clostridium botulinum</i>	Gram positives	Gastrointestinal tract, nervous system	Food poisoning (botulism)
<i>Clostridium tetani</i>	Gram positives	Wounds, nervous system	Tetanus
<i>Haemophilus influenzae</i>	Gram negatives	Ear canal, nervous system	Ear infections, meningitis
<i>Mycobacterium tuberculosis</i>	Gram positives	Respiratory tract	Tuberculosis
<i>Neisseria gonorrhoeae</i>	Proteobacteria ( $\beta$ group)	Urogenital tract	gonorrhea
<i>Propionibacterium acnes</i>	Actinomycetes	Skin	Acne
<i>Pseudomonas aeruginosa</i>	Proteobacteria ( $\beta$ group)	Urogenital canal, eyes, ear canal	Urinary tract infection, eye and ear infection
<i>Salmonella enteritidis</i>	Proteobacteria ( $\gamma$ group)	Gastrointestinal tract	Food poisoning
<i>Streptococcus pneumoniae</i>	Gram positives	Respiratory tract	Pneumonia
<i>Streptococcus pyogenes</i>	Gram positives	Respiratory tract	Strep throat, scarlet fever
<i>Treponema pallidum</i>	Spirochetes	Urogenital tract	Syphilis
<i>Vibrio parahaemolyticus</i>	Proteobacteria ( $\gamma$ group)	Gastrointestinal tract	Food poisoning
<i>Yersinia pestis</i>	Gram negatives	Lymph and blood	Plague