



Curriculum Units by Fellows of the Yale-New Haven Teachers Institute
2010 Volume III: Geomicrobiology: How Microbes Shape Our Planet

The Wonders of Bacteria

Curriculum Unit 10.03.01
by Haifa Abdel-Jalil

Introduction

Have you ever wondered what kind of organisms live in volcanic sulfur springs like at Yellowstone Park or in deep-ocean hydrothermal vents? Or questioned why the world is not filled with twigs, leaves, and undecayed bodies? Microscopic life covers nearly every square centimeter of Earth. These small organisms play a significant role in our lives. They are found in the air, on our skin, and deep beneath the surface thriving; even under extremely harsh conditions.

The smallest and most common microorganisms are Prokaryotes -unicellular organisms that lack a nucleus. They typically range in size from 1 to 5 micrometers. ¹ Biologists have divided prokaryotes into two different groups: the Bacteria and the Archaea. Although prokaryotes are physically different than eukaryotes (organisms whose cells contain a nucleus and membrane bound compartments) prokaryotic cells perform many functions that enable them to survive, reproduce, thrive and dominate the living world. Some prokaryotes achieve these functions under extreme environmental conditions such as high temperatures, or low pH.

Extreme halophiles, which are salt-loving archaea can live at very high salt concentration such as in Great Salt Lake and even the Dead Sea. Although eukaryotes are far more complex than prokaryotes; these conditions are intolerable for eukaryotes. Another group of archaea is the Methanogens, prokaryotes that produce methane gas. These archaea live in oxygen-free environments such as anoxic mud and the digestive tract of some animals, especially in the rumen of cows and other grazing animals. The methanogens use hydrogen and carbon to produce methane (CH_4). In the atmosphere, the methane reacts with oxygen to produce CO_2 . Without methanogens Earth would be a really different place and oxygen would make up a much greater percentage of the atmosphere.

A most fascinating group of bacteria, however, are the cyanobacteria. They were once classified as "blue green algae". These bacteria have an elaborate and highly organized system of internal membranes, which enables them to function in photosynthesis, a process by which cells convert energy of sunlight into chemical energy. Organisms that perform photosynthesis require sunlight. Therefore, they are only found in terrestrial and aquatic environments where sufficient light can penetrate. Cyanobacteria perform photosynthesis in a similar way to some eukaryotic algae and plants. Some types of cyanobacteria can transform molecular

nitrogen into a form usable by plants.

This unit will reveal many interesting and amazing facts about the role of bacteria –organisms that came into existence billions of years before us, in our lives and in natural ecosystems. Through this unit, students will expand their horizons by learning more about the wonders of bacteria.

Objectives

- Define bacteria and archaea and note the relationships between them.
- Describe the methods used to classify bacteria.
- Understand the structure of a bacterial cell.
- Describe the different styles of movement used by bacteria.
- Describe the metabolic diversity of prokaryotes and understand how this diversity plays a key role in maintaining the web of life on Earth.
- Understand the role of bacteria in natural ecosystems.
- Identify the ways that bacteria can cause diseases in humans.
- Specify how antibiotic resistance has come about, and describe ways that bacteria resist antibiotics.
- List ways that bacteria are helpful to humans.

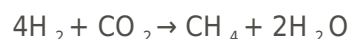
Evolution of Microbial Life

Earth has undergone a continual process of geological and physical change from the time of origin about 4.6 billions years ago. ² Microbes have always been able to adapt and cope with these changes. The dating of rocks based on the decay of radioactive isotopes and the fossilized remains of cells provides evidence for early microbial life. Stromatolites, which are laminated microbial mats built from layers of filamentous bacteria that became fossilized are abundant in rocks up to 3.5 billion years old or younger. ² An interesting fact about stromatolites is that filamentous phototrophic bacteria, which might be related to the green non-sulfur bacterium *Chloroflexus*, may have formed ancient stromatolites.

Ancient stromatolites were more likely composed of anoxygenic phototrophs rather than oxygen-producing cyanobacteria, since molecular oxygen did not appear until the evolution of oxygenic photosynthesis by cyanobacteria. Therefore, It has been concluded that the first cells may have been anaerobic autotrophs due to the lack of oxygen and abundance of hydrogen and carbon dioxide. These anaerobic autotrophs obtained their carbon from CO₂ and used electrons derived from sulfides, hydrogen or ferrous iron to reduce CO₂ to cellular material from H₂.



After the development of early forms of carbon and energy metabolism, microbial life underwent a long process of metabolic diversification; and as the different elements and nutrients were consumed and became limited; the competition for resources and natural selection led to the evolution of new forms of metabolism. Molecular evidence suggests that the ancestors of the modern day archaea and bacteria had diverged by 4.1–3.9 billion years ago and that the development of distinct metabolisms where early bacteria may have used hydrogen and carbon dioxide to produce acetate or ferrous iron compounds as a source of energy² (i.e., electrons) had also occurred. At the same time, archaea- developed the ability to use hydrogen and carbon dioxide as substrates for the production of methane.



The modern day Bacteria and Archaea had existed as the only forms on our planet for about 2 billion years before eukaryotes evolved (Madison, Martinko, Dunlap, and Clark 2009). Eukaryotes originated after the rise in atmospheric oxygen. Oxygen was also a factor in endosymbiosis. The endosymbiotic theory suggests that eukaryotic cells were created from a symbiosis between different prokaryote cells that had the ability to use oxygen and generate ATP. These prokaryote cells have evolved into the mitochondria that are now part of multicellular organisms. The other significant part of the theory states that other prokaryotic cells that were able to carry out photosynthesis have evolved into the chloroplast, which is the organelle necessary to carry out photosynthesis in plants and algae.

Classifying Prokaryotes

According to molecular analysis, scientists have grouped all living things into three domains. Eukarya, Bacteria, and Archaea. The domain Eukarya includes protists, fungi, plants and animals. The Bacteria are the larger of the two domains of prokaryotes and include a wide range of organisms with different life styles.¹ They live in fresh water, salt water, on land, and even in the human intestines. Bacteria are usually surrounded by a cell wall that protects them and determines their shape. Their cell wall contains a carbohydrate-rich substance called peptidoglycan, which makes them different from archaea which lack peptidoglycan.

The archaea are equally small as bacteria, they lack nuclei and have a cell wall that is chemically different from the cell wall of bacteria. They also have different membrane lipids comprised of ethers as opposed to ester lipids of bacterial and eukaryotic cell membranes. Also, the DNA sequences of key archaeal genes are more like those of eukaryotes. Archaea live in extremely harsh conditions and environments such as acid lakes or volcanic hot springs.¹

Bacterial Morphology

Prokaryotes come in have many different types and shapes, but the most common shapes are cocci and rods. Cocci are spherical cells that can exist in different arrangements; their shape can be useful in identification. For example, diplococcus is a pair of cocci that arises when cocci divide and remain together to form pairs. Long chains of cocci that result when cells stick after repeated division are characteristics of Streptococcus, and irregular shaped, grape -like clumps are characteristics of Staphylococcus. Rod -shaped bacteria are called bacilli. The shape of the rod's end often varies between species and may be flat, rounded, cigar-shaped, or bifurcated. ³ Another common shape for bacteria is the vibrio. Vibrios are comma-shaped bacteria that resemble rods. Spirilla are rigid, spiral- shaped bacteria. Spirochetes are another type of spiral-shaped bacteria, but they are flexible and have a unique internal flagella arrangement.

Prokaryotic cells exhibit a highly ordered intracellular organization. This organization is needed in order for the prokaryote cells to respond to the exterior environment and to respond to other cells. Also, prokaryotic cells must be able to transport materials from their surrounding environment into the cell and vice versa. In addition, they must protect themselves from the osmotic pressure, which is caused by movement of water freely across the cell membrane in response to concentration gradients. The cell wall, and the cell membrane fulfill these roles. Internal structures are also responsible for the growth and reproduction of bacteria. The cell wall is a structure that surrounds the plasma membrane; the periplasm, found only in gram-negative bacteria is the space that lies between the cell wall and the cell membrane. The cell wall gives the bacterial cell its shape and provides protection. In some bacteria, the cell wall is strong enough to withstand 375 pounds per square inch of internal osmotic pressure. ⁴

The cell walls and cytoplasmic membranes of bacteria contain peptidoglycan. It is an enormous molecule composed of amino acids and carbohydrates. There are two types of walls found in bacteria and easily identified using a method called Gram staining. Gram positive bacteria are bacterial cells that have a very thick peptidoglycan cell wall. It is about (25nm) and it is the thickness of this material that allows the cell wall to retain the crystal violet dye used in the Gram stain.

Gram-negative bacteria have a cell wall with a different structure. The cell wall is much thinner and there is an outer lipid layer. This layer can be dissolved with alcohol during Gram staining, which removes the dye and makes the bacteria appear as pink upon counterstaining with Safranin solution. It is important to mention that in addition to peptidoglycan, most cell walls of Gram-positive bacteria contain teichoic acids, large molecules composed of repeating units of sugar and phosphates (McKane and Kandel, 1996) which gives the cell a negative charge determining the type of substances attracted to and transported into the cell.

The cell membrane of bacteria encloses the cytoplasm. It is about 5nm thick, and consists of 40 percent phospholipids and 60 percent protein. The mosaic of phospholipids and protein are not cemented. The phospholipids are arranged in two parallel layers and represent the barrier function of the membrane. Proteins are embedded in this phospholipid bilayer. These proteins carry out important functions such as cell wall synthesis, and energy metabolism. Another significant function for the membrane protein is transportation of charged solutes such as sugar, ions, amino acids and nitrogenous bases. The cell wall transport system is highly specific, and it may require energy when it allows different concentrations of solutes to be established outside or inside of the cell against its concentration gradient.

The cytoplasm consists of the cytosol, a semi-fluid mass of amino acids, proteins sugar, vitamins, salt, and

ions. Suspended in the cytoplasm of all bacteria is a region of chromosomal material called the nucleoid. The genetic material of prokaryotes is carried on a single circular molecule of DNA that constitutes the cell's nucleoid. Most cells have one copy of the chromosomes. However, the chromosomes divide before cell division.

The size of the chromosome varies according to the species. For example, *Mycoplasma*, the smallest bacteria contains the smallest strand of DNA, which directs the synthesis of fewer than 1000 cell products, while *Escherichia coli*, which is found in the gastrointestinal tract of mammals has a chromosome that contain information for the production of about 4000 products. ⁴ Many bacteria contain a plasmid- small circular piece of DNA that can replicate independently of the chromosome. Some plasmids are used to transfer genetic information between bacteria and are significant in Genetic engineering. Ribosomes are another component of the cytoplasm. These are hundreds of thousands of spherical particles and they are the sites for protein synthesis.

Outside the cell wall and membrane, many bacteria have a gel-like layer called a capsule. Most capsules are polysaccharides or polysaccharides-protein complexes. A thick capsule provides protection to certain bacteria, and prevents some types of bacteria from dehydrating. Also a capsule might protect bacteria from being engulfed and destroyed by the body's white blood cells. Some bacteria form thick -walled endospores around their chromosomes, and a small piece of their cytoplasm when the cell is exposed to harsh conditions such as heat, radiation, chemicals or lethal agents. This structure does not grow or reproduce. These endospores do not produce new cells; instead they can survive for thousands of years. Endospores are the most totally heat resistant form of life. They can survive in boiling water at 100 C for several hours. For example, spores of the bacterium that causes botulism, a fatal food poisoning, can survive in food that has been subjected to insufficient heating.

Finally, bacteria have several structures that project through the cell wall to form surface appendages. The most common are the flagella and the pili. The flagella consists of a body, a hook, and a filament. It resembles a rigid corkscrew that spins, much like the propeller of a boat. The rotation of a flagellum has been measured to be as high as 300 revolutions per second. Therefore, flagellated bacteria are capable of very rapid movement. Pili are protein tubes that extend from the cell; they are shorter and thinner than flagella. They are only found in certain species of gram-negative bacteria. Pili do not play a role in motility, but they help in conjugation between bacteria, and attachment of bacteria to other surfaces, such as tissues of an infected person. ⁴

Movement

Many bacteria use flagella to move, the flagella turn and propel which make bacteria move. Bacteria may have a single flagellum or a clump of flagella. Other bacteria have flagella at both ends of the cell. Bacteria that lack flagella have other ways of movement. For example, myxobacteria produce a layer of slime; just like a slime trail which allows them to glide through it. Another kind of movement used by bacteria is corkscrew-like rotation; the spiral-shaped bacteria use this kind of movement. Bacterial motion is random, but sometimes bacteria that have flagella can move toward chemical nutrients, or away from a repellent such as poison. This behavior is called chemotaxis. ⁴

One of the most amazing types of bacteria is the magnetotactic bacteria (MTB). Blakemore (1975) was able to isolate a bacterium that lived in marine mud. This bacterium moved toward the geomagnetic North Pole. Since that time, many MTB have been isolated, and most of them range from cocci to spirilla. ⁵ These bacteria can

migrate along the geomagnetic field toward their favorable habitat. They contain nanometer -sized crystals of iron minerals such as magnetite (Fe_3O_4) or greigite (Fe_3S_4). These crystals are enclosed in a membrane and arranged in linear chains adjacent to the cell membrane. This magnetic moment causes motion that is parallel to geomagnetic field lines, helping the bacteria to swim toward high oxygen concentrations at the oxic-anoxic interface of water. Scientists have identified two different types of these bacteria: polar and axial. The polar variety swim in a preferred direction relative to the local field. Some polar bacteria in the northern hemisphere respond to high oxygen levels by swimming toward geomagnetic south. The axial varieties of MTB swim in both directions and rotate 180 degrees continuing to swim back and forth. ⁶

Reproduction

Bacteria are active microorganisms, under ideal conditions they are constantly reproducing, metabolizing, and growing in number and in size. Bacterial growth is described as the increase in population size and it can occur in different ways. The predominant mode of bacterial reproduction is called binary fission, which is a form of asexual reproduction that produces two daughter cells. During binary fission, the cell increases in size and doubles in length, and the cytoplasmic volume increases since it is filled with new ribosomes and enzymes. The cell duplicates its genome and divides its resources in half. Each daughter cell gets the genetic instructions and other cellular constituents that are needed to continue the cycle. Once the cell wall is completed, the daughter cell becomes independent. Each time the cell divides by binary fission it forms a new generation of cells. Some bacteria reproduce by budding, in which smaller cells are produced from the surface of the parent bacteria.

Bacteria have several ways of transferring genetic material or DNA that do not involve growth. For example, two living bacteria can bind together and transfer genetic material in a process called conjugation. During conjugation, one bacterium must have a specialized plasmid and pilus. The specialized pilus can bind to the recipient bacterium and form a conjugation bridge, which is a passageway that enables the bacterium to transfer genetic information. In order for this process to occur, one copy of the plasmid passes through the bridge (pilus) to the recipient bacterium. The cells will detach after the transfer of DNA. Conjugation pili are longer and fewer than the pili used for attachment. ⁷ Conjugation increases diversity in the population of bacteria. Other bacteria produce endospores that can remain dormant for years while waiting for conditions to improve. The ability of many bacteria to form spores make it possible for them to survive harsh conditions such as extreme heat, dryness, and lack of nutrients that would otherwise kill them.

Metabolic Diversity

All living organisms including bacteria need a constant supply of energy. Growth, movement, metabolism, and protein synthesis require a constant supply of energy. The processes of respiration (a.k.a. breathing) and fermentation, both release energy. Organisms that depend on the presence of oxygen in order to live are called obligate aerobes. For example, *Mycobacterium tuberculosis*, the bacteria that causes tuberculosis is an obligate aerobe. Some other bacteria cannot live in the presence of oxygen; in fact, they can be killed by it. This type of bacteria is classified as obligate anaerobes. *Clostridium botulinum*, which is found in soil is an obligate anaerobe. Another group of bacteria is classified as facultative anaerobes. These bacteria can live in the presence or absence of oxygen. The ability of these bacteria to switch between cellular respiration and fermentation means that they can live almost everywhere. For example, *E.coli* is a facultative anaerobe that can live in contaminated water, in sewage, or in the large intestine. ¹

Bacteriologists usually characterize an organism's nutritional source need according to the carbon source and

the energy source it requires for growth. Depending on their source of energy, and their use of oxygen, prokaryotes can be divided into several types. Heterotrophs utilize organic compounds to get the carbon, necessary for growth. Other prokaryotes are autotrophs; they make their own food/biomass from inorganic carbon or carbon dioxide (CO_2) molecules. Autotrophs can be further divided into two categories. The photoautotrophs derive energy from sunlight through the process of photosynthesis, using light energy to convert carbon dioxide and water to organic carbon compounds and oxygen. Therefore these bacteria are found in areas where light is plentiful such as the surfaces of lakes, streams, or oceans. Photosynthesis occurs in the green sulfur bacteria, the purple sulfur bacteria, and cyanobacteria. Cyanobacteria are the most diverse and largest group of photosynthetic bacteria. They have chlorophyll a, and use phycobiliproteins as accessory pigments. ² Cyanobacteria are found in some fresh water, salty water, or even on land.

The chemolithoautotrophs do not require light as a source of energy; instead they use chemical reactions that involve the oxidation of inorganic compounds or chemicals such as hydrogen sulfide, ammonium, nitrites, sulfur, hydrogen (as H_2) or iron. Oxidation is the loss of electrons or hydrogen atoms in a chemical reaction that result in the release of energy. Chemolithotrophic bacteria use oxygen or other electron acceptors in respiration in order to make ATP and get their energy. Other types of bacteria oxidize nitrogenous compounds to get their source of electrons. Nitrifying bacteria are an example of this group. These bacteria can live in soil or aquatic environments. They are significant because they play a role in the process of nitrification which oxidizes ammonia into nitrate. The chemoheterotrophs use organic compounds as a source of both energy and carbon. Many bacteria and some archaea are examples of this type.

Sulfur oxidizing bacteria

The sulfur -oxidizing bacteria are chemolithotrophs, they use sulfur oxidation as a means of gaining energy or electrons. The sulfur-oxidizing bacteria can oxidize hydrogen sulfide, polysulfides, and elemental sulfur. These compounds can be found in nature under various aerobic and anaerobic conditions. Sulfur -oxidizing bacteria can be found in geological materials such as volcanic gases and deposits of elemental sulfur. In most cases, oxygen is the preferred electron acceptor. When certain bacteria derive energy from the oxidation of sulfide, the populations of bacteria grow, and in return these bacteria provide food for nearby animals such as bivalve mollusks, which actually allow sulfur-oxidizing bacteria to reside within their gills. Some of these bacteria live in mats on rocky surfaces and on soft sediments, providing a source of food for crabs and bivalves. Sulfide may be oxidized to elemental sulfur by species of *Thiothrix* and anaerobically by the purple sulfur bacteria. Some eukaryotes have sulfide binding proteins, which allow them to transport sulfide and oxygen to the bacteria through specific organelles such as the trophosome found in hydrothermal vent tubeworms.

Sometimes natural gas deposits contain high concentrations of hydrogen sulfide and thus, create an environment where sulfur-oxidizing bacteria can thrive. Sulfur oxidizers are also found in low pH environments such as acid mine drainage waters. These acid-loving sulfur-oxidizing bacteria called (acidophiles) metabolize sulfur in sulfur-rich coals. When sulfur-rich coals or minerals like pyrite (fool's gold= FeS_2) are exposed to oxygen, the acidophilic, sulfur-oxidizing bacteria can convert sulfur to sulfuric acid and create an environment with very low pH (~1-2 or lower). *Thiobacillus* species are often found in both marine and fresh water sediments, other species of sulfur-oxidizing bacteria such as *Beggiatoa* can grow on sulfide, which is confirmed by the presence of intra-or extra sulfur granules in the cell. ⁸

Importantly, hydrogen sulfide is normally toxic to aerobic plants and animal tissues, with the exception of ruminant animals, and whenever sulfur is generated, a specialized micro flora develops that is capable of oxidizing the sulfide into elemental sulfur.



The oxidation of sulfur is shown below



Methanogenic archaea

Methanogens are anaerobic archaea that synthesize organic compounds in a process called methanogenesis that produces methane. Methanogenic archaea thrive in swamps, hot springs, fresh water, and marshes. They are also found in the rumen, an expanded upper compartment of the stomach of cows that contains regurgitated and partially digested food. Methanogenic archaea use hydrogen and carbon to produce methane (CH_4), and the methane can react with oxygen in the atmosphere producing CO_2 , thereby reducing the amount of atmospheric oxygen.

Bacteria and Diseases

Much of our knowledge about bacteria is the result of the study of diseases that they cause in humans. Pathology is the scientific study of diseases. Bacteria can cause diseases by producing poisons called toxins. These toxins are of two types. The first type is known as exotoxin. Exotoxins are made of protein and released by living Gram-positive bacteria, which secrete these toxins into the surrounding environment. The second type of toxin is called endotoxin, which are made of lipids and carbohydrates, and are associated with the outer membrane of Gram-negative bacteria such as E.coli. ⁹ These toxins are not released until the bacteria are dead and once they are released they cause body aches, fever, weakness, and damage to the vessels of the circulatory system. Another method of causing diseases used by bacteria is destroying body tissues. Some bacteria adhere to cells and secrete digestive enzymes, which further attack the tissues. An example is Streptococci which produce a blood-clot- dissolving enzyme that enables these infectious bacteria to spread into other tissues.

Antibiotics

Antibiotics are chemicals produced by microorganisms [and plants] that selectively kill or inhibit the growth of other microbes. Antibiotics interfere with various cellular functions. For example, penicillin interferes with cell wall synthesis, while tetracycline interferes with bacterial protein synthesis. Many antibiotics are derived from chemicals that bacteria and fungi produce, while others are synthesized in the laboratory such as the sulfa drugs. It is important to say that the most effective agents were isolated from the mold Penicillium. Another significant producer of antibiotics are the streptomycetes. They provide most of the world's antibiotics. Over 50 distinct antibiotics have been produced by streptomycetes. It is also important to mention that antibiotic-producing organisms are resistant to their own antibiotics, but remain sensitive to antibiotics produced by

other species of streptomyces.

Streptomyces is a large group of filamentous bacteria that resemble fungi. They are part of the Actinomycetes family. These filamentous bacteria form spores called conidia. The spore-bearing structures are called sporophores. They are usually pigmented and mature colonies are colored, which makes them easier to detect on agar plates. Streptomyces are capable of metabolizing different compounds such as sugar, alcohol, amino acids and some aromatic compounds. They also produce extra-cellular enzymes to help them digest starch and cellulose. Although they can be found in aquatic habitats, these bacteria usually inhabit the soil, and are responsible for the earthy smell of the soil due to the secretion of geosmins. Streptomyces are obligate aerobes. They prefer alkaline and neutral soil, and can be found in well-drained soil such as sandy loams or soils covering limestone. Streptomyces can also degrade organic matter and, therefore, found in compost piles.

When a population of bacteria is exposed to an antibiotic, the bacteria that are most susceptible to antibiotics die first. The therapeutic effectiveness depends on the sensitivity of the pathogen to the drug. Other microbes may vary in their responses. Antibiotic resistance may develop in microbes within the population. Antibiotics do not create resistant cells, but selectively favor the survival and reproduction of the drug-resistant strains. Antibiotic resistance occurs due to mutations in the pathogen's chromosome. In this case, most of the sensitive bacterial cells are killed or inhibited, but a few resistant cells are uninhibited and continue to grow. The prolonged exposure to the antibiotic prevents the sensitive cells from repopulating the area allowing the resistant microbes to become predominant. The second reason for antibiotic resistance is the direct transfer of R-factor plasmids, a small, closed-loop molecule of DNA, from the antibiotic-resistant to the sensitive recipients through the process of conjugation. R factors carry genes for multiple resistances, fortifying the bacterial recipient with protection from a number of drugs. ⁴ Bacteria use many mechanisms to resist antibiotics. These mechanisms include the ability to either inactivate or destroy the antibiotic by producing extra cellular enzymes, or by decreasing the antibiotic uptake by modifying or reducing the permeability of the cell membrane to drugs or by cross resistance, whereby a single cellular modification may provide resistance to all tetracycline, for example.

Antibiotics are also added to animal feed of pigs; cattle, chickens and other farm animals to encourage meat production, but this practice has promoted the survival of antibiotic-resistant microorganisms. Most of this is the R factor type that unfortunately might transfer antibiotic resistance to human pathogens when these animals shed, or when they are slaughtered. For example, the antibiotic-resistant Salmonella can be transferred from livestock to humans. Many countries such as the Netherlands passed a law banning the use of antibiotics that are used to treat humans as a growth-boosting supplements in animal feed.

Useful Bacteria

Most humans think of bacteria as harmful organisms causing diseases and threatening our lives. In fact, most bacteria are harmless and many are beneficial and vital to our living world. Some of the following are benefits of bacteria.

1. Nitrogen Fixation

Animals depend directly or indirectly on plants for energy, and plants depend on bacteria to get nitrogen.

Nitrogen is necessary for plants to build amino acids and proteins. But plants cannot get the nitrogen directly from the atmosphere; they depend on bacteria to convert the nitrogen into a form that can be used by plants. This process is called nitrogen fixation. Bacteria in the soil can change nitrogen into ammonia ((NH_3)), some of these bacteria are free living, while others such as Rhizobium bacteria can form a symbiotic relationship in which soy beans provide nutrition for the bacteria, while bacteria convert nitrogen into ammonia. Nitrogen is also cycled by processes of bacterial nitrification. The most important groups of microorganisms involved in this process are the chemolithoautotrophic ammonia- and nitrite-oxidizing bacteria in the family Nitrobacteraceae. ¹⁰ Nitrosomonas includes a number of genera that oxidize ammonia to nitrite, and then Nitrobacter oxidizes the nitrite to nitrate.

2. Production of food and beverages.

Bacteria are used in the production of a wide variety of food and beverages. For example, Lactobacillus and Streptococcus thermophilus are used in making yogurt through fermentation. Fermentation is a process by which energy is released from food molecules in the absence of oxygen. Bacteria produce lactic acid as a waste product in the process of fermentation, and this is used for the production of a variety of food and beverages such as yogurt, kimchi, sauerkraut, buttermilk, pickles and cheese. Also, lactic acid bacteria produce antimicrobial substances, sweeteners, aromatic compounds, vitamins, and useful enzymes.

3. Genetic engineering and medicine.

Bacteria can be transformed by using recombinant DNA. The foreign DNA can be joined to a small DNA molecule known as a plasmid. Plasmids are small, circular, extra chromosomal DNA molecules that replicate independently. A plasmid is very useful in DNA transfer, because it has a DNA sequence that helps promote plasmid replication. Therefore, if the plasmid containing the foreign DNA manages to get inside a bacterial cell, this sequence ensures that it will be replicated. ¹

Classroom Activities

The following activities have been designed to guide and help the students understand the concepts that have been introduced, and to encourage students to use critical thinking, draw conclusions and make a connection.

Activity 1

Bacterial types and structure activity sheet

In this exercise, students will be given a copy from the biology-coloring book. ¹¹ This copy includes illustrations for three sections: one that shows common bacterial shapes, one that shows bacterial structure and the a third that describes bacterial reproduction. Students will use colored pencils to identify the different shapes of

bacteria, the parts and the process of reproduction by binary fission. The illustration is labeled with different letters and the students have to follow the instructions for coloring, which dictate that the same letters should be colored in the same colors.

Objectives

Students will be able to identify the three major different shapes of bacteria; understand the basic structure of bacterial cells and know that bacteria reproduce asexually by binary fission.

Materials needed

- A copy of the illustration sheet
- Coloring pencils
- Glue sticks
- Construction paper

Procedure

1. Students will use different colored pencils to color the bacterial types, bacterial structures and bacterial reproduction including the key.
2. Use the glue stick to glue the finished work sheet onto a piece of construction paper and post it on the classroom wall.

Evaluation: Students will be given a table that includes the basic structure of bacteria; students should fill in the blanks, information about the location of the structure and its function. Additionally, they will write a paragraph to describe the different shapes of bacteria and how they reproduce.

Activity 2

Bacteria and population growth

Bacteria are unicellular prokaryotes, and they are found almost everywhere on the surface of the earth. They are divided into different groups according to their characteristics. Bacteria divide asexually by binary fission producing identical cells, with the right temperature, suitable environment, and plenty of food bacteria can divide into two cells in about every 20 minutes to an hour.

Objectives

Students will be able to graph the data for bacterial growth, and predict the type of growth.

Materials needed

- Pencils, graph paper, data for bacterial growth

The following data show the number of bacteria in a petri dish

Hours	1	2	3	4	5	6	7	8	9
# of Bacteria	1	2	4	8	16	32	64	128	256

Use what you have learned about bacterial growth and reproduction to answer the following questions.

- 1- Name two ways by which bacteria reproduce.
- 2- Find the ratio of bacterial growth between two consecutive hours such as 4 and 5.
- 3- Predict the number of bacteria during hour 10.
- 4- Use graph paper to plot time vs. number of bacteria. This growth plot produces a curve, what is the name of this curve?

Activity 3

Controlling Bacterial Growth

Antibiotics are chemical substances that inhibit bacterial growth. Living organisms can produce some antibiotics. Other antibiotics are synthesized in the laboratory.

Objectives

Students will learn the basic techniques for culturing bacteria on agar plates. They will also test the effectiveness of certain antibiotics on bacterial growth- and measure the zone of growth inhibition in the presence of antibiotics.

Materials needed

- Antibiotic disks (penicillin, streptomycin, tetracycline).
- Non -pathogenic culture of Escherichia coli
- Sterile forceps
- Metric ruler
- Sterile nutrient agar plates
- Sterile filter-paper disk

- Transparent tape
- Glass-marking pencil
- Inoculating loop or sterile cotton swabs
- Safety goggles, aprons, and gloves.
- A beaker with diluted Clorox bleach (10%) bleach)
- Bunsen burner

SAFETY

- 1-Students must follow the lab safety procedure when performing this lab. All students must wear safety goggles, apron, and gloves. Also they should tie back loose hair.
- 2- Students must clean their lab station and handle their materials carefully.
- 3- Although this bacterial culture is non-pathogenic, students should be careful when they streak the agar plates with the bacterial culture, and make sure that they do not re-open the plates after they are inoculated with bacteria, closed and taped.

Consult and follow the safety guidelines of your institution.

Procedure

- 1- Use a glass -marking pencil to mark the bottom of a sterile petri dish. Divide it into four quadrants and label each quadrant as follows:

Quadrant one: control

Quadrant two: Penicillin disk

Quadrant three: Streptomycin disk

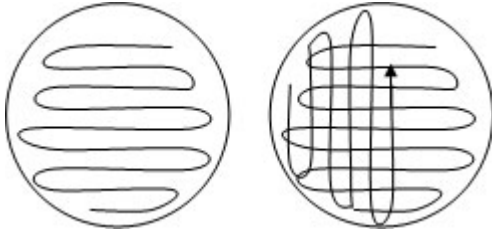
Quadrant four: tetracycline disk

Make sure that you write your initials and the date near the edge of the dish.

- 2- Insert a sterile cotton swab into the bacterial culture; return the bacterial culture to the test tube rack.

Open the sterile agar plate slightly, and place the tip of the cotton swab on the top center of the

- 3- the agar and streak the agar in a back and fourth motion until you cover the entire plate as shown in the Fig. Be careful not to dig deep into the agar.



- 4- Place the cotton swab in the beaker with the diluted Clorox bleach.
- 5- Use a sterile forceps to pick up the penicillin disk and carefully place it in the center of quadrant 2.
- 6- Pass the forceps back and forth through the flame of a Bunsen burner several times to sterilize the forceps. Be careful to not lean toward the Bunsen burner.
- 7- Repeat steps 4 through six with the remaining antibiotic disks in quadrants 3 & 4, and remember to sterilize the forceps after each step.
- 8- In quadrant 1, place a filter -paper disk soaked in distilled water.
- 9- Use transparent tape to tape the petri dishes closed. Turn the dishes upside down and incubate them for 48 hours at 37 °C .
- 10- Observe the petri dishes after 48 hours. Hold the dishes to the light to see the zone of inhibition clearly.
- 11- Use a metric ruler and measure to the nearest millimeter the size of the clear zone around each antibiotic disk.
- 12- Record your data in the following table. If there is no inhibition zone, record the measurement as zero.

Type of Antibiotic Disc	Size of zone of inhibition (mm)

Give the petri dishes to your teacher for proper disposal.

Performance Assessment

I. Analysis questions

1. Why do you think it is very important not to open the sealed petri dishes?

2. What was the control in this experiment? Why is it significant to use a control?
3. Which antibiotic disc was most effective in inhibiting the bacterial growth? Why?
4. Compare the colonies in each quadrant, and record your observations.

II. Lab report

Students will write a complete lab report for the experiment. They will be graded according to a scoring rubric.

Activity 4

Gram- staining technique lab

Hans Christain Gram is the Danish microbiologist who developed the Gram staining technique. This technique is one of the most significant techniques used in the field of microbiology. Gram staining provides a key for identifying and classifying bacteria- because bacteria are transparent and - therefore difficult to see under the compound microscope. Staining the specimens can increase visibility and help identify the microbes. Staining involves first heat -fixing the bacteria to a slide, then saturating the slide with a dye that reacts with various parts of the cell to stain. Heat fixing and staining may kill the bacteria, but the shape of the organism will be preserved. Methylene blue and crystal violet are commonly used stains. These dyes are positively charged and- therefore bind to the negatively charged polysaccharides and proteins on the cell wall surface, and on the inside of bacterial cells.

Gram staining can divide bacteria into two groups: Gram- positive and Gram-negative.

Objectives

Students will learn the Gram staining technique and distinguish between Gram-positive and Gram-negative bacteria.

Materials needed

- Bacterial culture
- Crystal violet -Ammonium Oxalate solution
- Gram's Iodine solution
- Counterstain -Safranin
- Decolorizing alcohol
- Apron, safety goggles, and gloves.
- Inoculating loops
- Distilled water
- Bunsen burner
- Slides

Procedure

Students should review all safety procedures and the proper aseptic techniques before they start working. Students should wear safety goggles, aprons and gloves.

Smear preparation

1. Sterilize the inoculating loop by holding it in the flame of the Bunsen burner for a few seconds, then allow it to cool for ~ 15 seconds.
2. Place a loopful of distilled water in the center of a clean slide
3. Take a small amount of the bacterial culture by using the inoculating loop and place it in the center of the water drop.
4. Mix the culture with the distilled water by using a circular motion and spread it in the center of the slide.
5. Sterilize the inoculating loop again and allow the slide to air dry.
Fix the slide by passing it 2-3 times over the Bunsen burner, and make sure that the smear side is up. Make sure that you do not expose the slide to excessive heat so that the slide will not break or be damaged.

Staining the culture

7. Place about 5 drops of the crystal violet stain to flood the culture smear by using a dropper, and allow it to stand for about one minute, then gently rinse the slide off with tap water for about 5 seconds and drain.
8. Place 5 drops of the iodine solution onto the culture and allow it to work for about 1 minute, then gently rinse the slide and drain by shaking off the excess water.

Decolorizing the stain

Alcohol should be used; this step is very important and the procedure is especially sensitive to it.

9. Place 5 drops of 95% alcohol in a way that flows down the slide from one end and continue until the solvent alcohol is no longer colored, if the smear is too heavy, you might have to repeat the process one more time. If you decolorize too long, the crystal violet stain may be completely removed.
10. Gently rinse the slide with water, and pat dry with lint-free paper, make sure that you pat it gently so that the smear will not rub off.

Counter staining the smear

11. Place about 5 drops of the safranin solution on the smear and allow it to stand for about 25 seconds
12. Gently rinse the safranin away with water and dry it gently by using bibulous paper to remove excess water

Observing the slide

13. Examine the slide under an oil immersion (100x) objective lens.

Gram -positive bacteria will appear blue to purple, and gram-negative bacteria will decolorize but retain the reddish or pinkish color of the counterstain.

Evaluation

Students will write a lab report including the procedure and results including an illustration for the shape, color and classification of the bacteria that have been observed.

Activity 7

Bioluminescent Bacteria

There are some bacteria found in seawater, marine sediments, and the guts of marine animals that emit light. Some fish such as flashlight fish, anglerfish and also bobtail squid have evolved and developed the ability to use these microbes for their own benefit and establish a symbiotic relationship. These animals have developed special organs that provide bioluminescent bacteria with a source of food and a place to live, and in return these animals can use the glowing bacteria as a means to camouflage and protect themselves from their predators, hunt for food, or even attract their mates.

Objectives

Students will be able to isolate luminescent bacteria from seawater and grow them on seawater complete agar.

Students will observe the luminous colonies in a dark room, and learn about bioluminescence.

Recipe for SWC (seawater complete agar can also be ordered from media supply catalogs.)

- 750 ml artificial seawater sea.
- 250 ml distilled water
- 5 grams Bacto peptone
- 3 grams yeast extract
- 3 ml glycerol
- 15 grams agar

For storage vials, add 1.0 g of CaCO_3 /liter

Mix the media in a container large enough to hold 1 L of media, and boil the media for ~ 30 minutes making sure that it does not boil over. Allow it to cool down enough to be touched (50-60 degrees C). Then pour media into sterile petri dishes (~15 mL media per standard petri dish)-Agar will solidify once the temperature falls below 40 degrees C.

Materials needed

- 5-10 mL sample of seawater
- 4-20 plates of sea water complete medium
- Glass spreading rod
- Incubator
- Sterile tooth picks
- Pipettes
- Gloves, safety goggles and aprons

Procedure

1. Collect a sample of about 5 - 10 ml of seawater to use as inoculum.
2. Take 2 plates of SWC agar medium and use a pipette to measure 0.1 ml of the seawater inoculum and pour it on the center of a plate. Repeat for the second plate.
3. Pour 0.2 ml of seawater inoculum onto the third plate. Repeat for the fourth plate.
4. Use the glass-spreading rod, spread the inoculum thoroughly over the surfaces of the plates. Allow the sample to be absorbed by the medium.
5. Invert the plates and incubate at 20 degrees C.
6. Examine the plates after 24 hours in a dark room. Watch for 'spreaders'-bacteria that can spread across the plates contaminating and engulfing other bacteria.
In the dark room, (with red light) use sterile toothpicks to pick 2 or 3 isolated luminous
7. colonies and transfer to fresh SWC plates, then use an inoculating loop to streak the fresh SWC plates to get isolated colonies of the luminous bacteria. Again, watch out for spreaders.

Analysis Questions

1. Describe the chemical reaction of the luciferase enzyme that bioluminescent bacteria use to emit light. What is the role of oxygen in this reaction?
2. Describe the colonies. What do they look like?
3. Where can you find these bacteria?
4. Define Quorum Sensing. Why is it important?
5. Describe the symbiotic relationship between bioluminescent bacteria and other organisms.
6. Do you think these bacteria can be beneficial in the medical field? Why?

Activity 8

Observing Cyanobacteria under the microscope.

Cyanobacteria are oxygenic phototrophs; they are widely distributed in terrestrial, fresh water, and marine habitats. Cyanobacteria can be more tolerant to environmental extremes such as salty lakes, while other species can be found on the surfaces of soil or rocks. Cyanobacteria can be unicellular or filamentous; some of these filaments are branched. They get their name from phycocyanin, a bluish pigment used to capture light for photosynthesis. They also contain chlorophyll a, which is the photosynthetic pigment used by plants for photosynthesis. Cyanobacteria are responsible for fixing a significant amount of carbon dioxide and for producing oxygen on Earth.

Objectives

Students will be able to make a wet mount slide.

Observe the shape and structure of cyanobacteria under the microscope.

Material needed

- Microscope
- Slides
- Forceps
- Pond water or salt marsh water sample
- Pipette

Procedure

- 1- Use a pipette to take a drop from the sample water and place it in the center of the slide. (Make sure that you can get some of the green stuff on the slide by using the forceps).
- 2- Gently place a cover slip on the sample, avoid making air bubbles.
- 3- Use the low-power objective lens to locate the cyanobacteria under the microscope. Turn the coarse adjustment knob until the cells come into focus.
- 4- You can switch to the medium power (40X) objective to view the slide and see more details of the shape of the cyanobacteria. (cyanobacteria will look like green strings).
- 5- Observe the cells, draw and label the appropriate parts if you can. Write down the magnification power.
- 6- Clean the slides carefully, and turn off the microscope

Performance Assessment

- 1- Draw and label the slides.
- 2- Describe the shape of the cyanobacteria cells.
- 3- Describe the color of the cyanobacteria cells.
- 4- How are these cells similar to plant cells? How are they different?
- 5- What is the advantage of preparing a wet mount slide and viewing the slide under the microscope?

Activity 9

Design a brochure

Objectives

Students will use the Internet to research and learn about the role of bacteria in our society, and the different applications of technology regarding the use of bacteria.

There are many topics and avenues that you can select from such as

- Genetic engineering (Bacteria -produced Medicine)
- Environmental Effects, cleaning toxic waste and oil spills
- Autotrophic bacteria
- Sulfide-dependent bacteria
- Biological warfare
- Role of bacteria in the food industry.
- Bacteria and mining.

- Antibiotic resistant bacteria.

Evaluation

Students will create a brochure or construct a poster to share their findings with their classmates. Students will be evaluated according to the following rubric

Category	Developing	Proficient	Exemplar
Organization	Many sections do not have titles and are not clear	Almost all sections has titles and beginning, middle, and end	Each section has a title with beginning, middle, and end
Writing and Grammar	Fonts are not clear Many errors in sentence structure, capitalization and punctuation	Most fonts are clear, minor errors in sentence structure capitalization and punctuation	All fonts are clear. No errors in sentence structure, capitalization and punctuation are correct
Content accuracy	Fewer than 80% of facts and science concepts are accurate	More than 80 % of the facts and the science concepts are clear and accurate	All facts and science concepts in the brochure are clear and accurate
Graphics and pictures	Graphics are randomly chosen, and do not relate to the topic	Graphics relate to the topic, but can use better graphics	Graphics relate to the topic, good mix of pictures, interesting and eye catching
Knowledge Gained	Only several students can understand the brochure and answer questions	Most Students can read the brochure and answer questions related to the facts	Students can read the brochure and answer all questions related to the facts
Sources	One source, and the source is not accurately cited	Used at least two sources. Sources are accurately cited with minor spelling errors	Used at least two sources. Sources are accurately cited with no spelling errors

Implementing District Standards

Grade 10 Core themes, Content Standards and Expected performances

Strand IV -Cell chemistry and Biotechnology. Content standard 10.2 - Microorganisms have an essential role in life processes and cycles on Earth.

This unit will be taught to a 10th grade biology class of about 80 students. The unit will be a part of the curriculum that focuses on microorganisms, classification, and organisms' interdependence.

The students come from different backgrounds and have mixed abilities and skills. Hill Regional is a Magnet

school that focuses on science and business. Many of my students would like to peruse a career in science; therefore they like to be challenged and want to expand their knowledge, while others need more attention and focus on basic skills. Hence, the learning needs vary by students; a variety of instructional strategies are essential in meeting their needs. These strategies include cooperative learning, modeling, observation, lecture and note-taking, lab work, reading for information and the use of technology.

References Used

- ¹ Miller, Kenneth and Levine, Joseph. Prentice Hall Biology.(Boston, Massachusetts: Upper Saddle River, New Jersey, 2006).
- ² Madigan, Michael T and Martinko, John M and Dunlap, Paul V and Clark, David P. Brock Biology of Microorganism (Pearson Education, Inc, 2009).
- ³ Willey, Joanne M and Sherwood, Linda M and Woolverton, Christopher J. Prescott's Principles of Microbiology. (New York, NY: McGraw Hill 2009).
- ⁴ McKane, Larry and Kandel, Judy. Microbiology Essentials and Applications. (The McGraw -Hill, Inc 1996, 1985).
- ⁵ Edited by J.C. Fry, G.M Gadd, R.A. Herbert, C.W. Jones and I.A. Watson-Craik. Microbial Control of Pollution. New York, NY: Press syndicated of the university of Cambridge, 1992.
- ⁶ Simmons, Sheri L. Bazylnski, Dennis A. Edwards, Katrina J. " South seeking Magnetotactic Bacteria in the Northern Hemisphere." Science 311(2006): 371-372.
- ⁷ Pommerville, Jeffrey C. Alcamo's Fundamentals of Microbiology.(Sudbury, Massachusetts: Jones and Bartlett Publisher, Inc 2007).
- ⁸ Neelson & Stahl. Microorganisms and Biochemical Cycles: What can We Learn From Layered Microbial Communities, 4-31.
- ⁹ Holt, Rinehart and Winston. Modern Biology. (Austin, Texas: Holt, Rinehart and Winston 2002).
- ¹⁰ Hans G. and Bowien, Botho. Autotrophic Bacteria. (Madison, Wisconsin: Science Tech Publishers, 1989).
- ¹¹ Alcamo, Edward. Biology Coloring Workbook. (New York: Random House, Inc., 1998).

Web Sites

1. Hagedorn, Charles. " Biogeochemical cycles". Spring 2010. Available from http://filebox.vt.edu/users/chagedor/biol_4684/index.html Accessed on June 29, 2010.
2. Dept of Ecology and Evolution SUNY Stony Brook. " Hot Vents". May 2006. Available from <http://life.bio.sunnysb.edu/marinebio/hotvent.html> Accessed on June 29, 2010.
3. Cyanobacteria: Life History and Ecology". Available from <http://www.ucmp.berkeley.edu/bacteria/cyano.html> Accessed on April 4, 2010.

<https://teachersinstitute.yale.edu>

©2019 by the Yale-New Haven Teachers Institute, Yale University

For terms of use visit <https://teachersinstitute.yale.edu/terms>