EXERCISE 1
THE MICROBIAL WORLD

INTRODUCTION

Microbiology is the study of very small organisms. The various fields of microbiology fall into the same categories as general biology: ecology, genetics, development, cell biology, and so forth. The kinds of organisms microbiologists study fall into several kingdoms of all three domains, and there is substantial diversity among them. They can belong to three Domains: Eukarya, Prokarya, and Archaea. They can also belong to many kingdoms. For example, eukaryotic microbes may include protists, fungi, and even some parasitic animals.

By definition, microorganisms are not visible with the naked eye, and must be viewed with a microscope. Because of their small size, bacteria and other microorganisms are not usually observed with low-power or dry objectives. Instead, they frequently are stained and observed with oil-immersion objectives, which have a magnification of about 100x. This exercise will give you experience in using a compound microscope with oil-immersion objectives.

The purpose of this exercise is for you to become familiar with:

- the various kinds of organisms that microbiologists study
- the basic structures of prokaryotic and eukaryotic cells
- the morphologies and sizes of various microorganisms
- the care and use of the oil-immersion compound microscope

TECHNIQUE:

Observe each of the prepared specimens with the 10x, 40x, and, if needed, the100x oil-immersion objective. Record the size and shape of the organisms in drawings, and include as many details as you can . You will also be responsible for any information given about the organisms.

Where possible, I have included pictures of the organisms so that you will know what to look for. These pictures **do not** substitute for finding the organisms and identifying the features yourself!

Protozoans

Protozoans vary greatly in size, but they look distinctly different from host tissues.

Entamoeba histolytica

E. histolytica causes amoebic dysentery. Probably half a billion people worldwide are infected, and 100,000 die each year. There are about 3-5,000 cases reported each year in the US. ***E. histolytica*** can from cysts that can easily be passed on in contaminated food or water. The trophozoite phase emerges from the cyst in the small intestine, then travel to the large intestine where they can invade host tissue or live in the lumen of the large intestine. If they invade host tissue, using a proteolytic enzyme, they can invade the bloodstream and travel to the liver, lungs, or brain where they form abcesses. Only a few strains of ***E. histolytica*** are pathogenic.

Leishmania donovani

This organism causes a form of leishmaniasis called kala-azar, and is usually found in parts of China, India, the Mediterranean, the Sudan, and Latin America. The reservoirs are typically dogs and rodents, and the protozoans are spread by sand flies as the vector. The flagellated cells invade the endothelial cells ofthe liver and spleen. They block the reticuloendothelial system, and cause anemia, toxicity, and enlargement of the spleen and liver. It is often fatal unless treated.

Trypanosoma gambiense (***T. brucei gambiense***)

This organism causes African sleeping sickness. The reservoir is cattle and wild animals; the tsetse fly is an intermediate host and vector. The flies transfer the protozoa to humans by biting them. The organisms multiply in the bloodstream, and ***T. gambiense*** invades the central nervous system. The disease is usually fatal in 2-3 years. The protozoans are about 15-30 µm long.

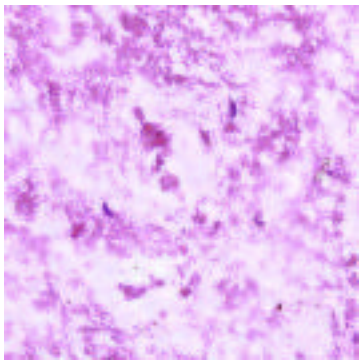
Plasmodium falciparium

One of the four species of ***Plasmodium*** that cause malaria. Malaria is most common in the tropics,where it may infect as many as 100 million people. It kills about 1,000,000 people each year. There are about 1,000 cases of malaria in the U.S. each year, mostly among people who have recently been in the tropics. There were however, at least 3 cases of locally acquired malaria in Michigan in 1996. Some people are naturally resistant to ***P. falciparium***; their genes contain one allele for hemoglobin S.

The life cycle of a ***Plasmodium*** is shown in the text in Figure 39.19. Ronald Ross received the Nobel Prize in 1902 for determining it. The sporozoite stage is injected into an animal by a mosquito, where it invades the liver and goes through rounds of reproduction. Then, it invades the red blood cells and prepares for meiosis. The premeiotic cells make gametes after they have been ingested by a mosquito. The gametes fuse, and give rise to a series of cells that eventually generate sporozoites.

Toxoplasma gondii

This protozoan requires cats to complete its life cycle; house cats are usually responsible for transmission to people. Cysts are transmitted by inhalation or by mouth. Pregnant women who become infected can transmit the protozoan to the fetus, in which it can cause birth defects or death. Pregnant women are usually discouraged from changing cat litter or other activities that might bring them into contact with cat feces.



Balantidium coli

This is the only parasitic ciliate known to infect humans. It inhabits the colon, and produces symptoms similar to those of ***E. histolytica***. It is common in pigs, which are often carriers. It does little harm in humans. It bears a vague resemblance to ***Paramecium***, and is about 50-100 µm long, and about 35-75 µm wide, and is covered with cilia.

Giardia lambia

This parasite causes severe gastrointestinal distress known as "beaver fever" or "backpackers' diarrhea". It was discovered by van Leeuwenhoek, but has become a health problem in the US only in the last 25 years. It has rapidly spread to even the most remote water supplies. It causes about 30,000 cases of epidemic diarrhea in the US each year, and between 5 and 10% of the population are carriers.

The organism is passed on in the water as cysts, and it may infect humans as well as domestic and wild mammals. The trophozoite stage emerges from the cyst in the duodenum, and the trophozoites attach to the intestinal mucosa where they can cause severe diarrhea, cramps, and gas.

The "eyes" of the parasite are actually nuclei, and the organism has bilateral symmetry. It attaches to the lining of the intestine with sucking disks. Interestingly, it seems to lack most organelles associated with eukaryotes.

trophozoite

Giardia lambia

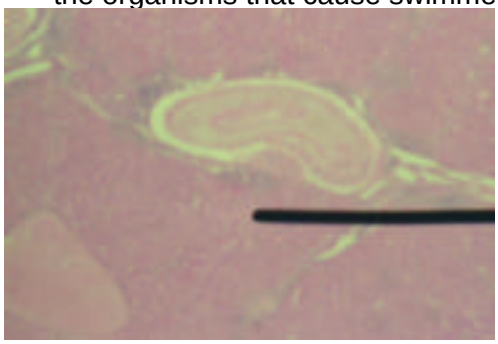
cyst

Animals

Remember that animals are multicellular, and very thick. The preparations you will observe are thin sections through host tissue, and you will usually only see a portion of the parasite. You will probably not see intact organisms, but should be able to see sections through them.

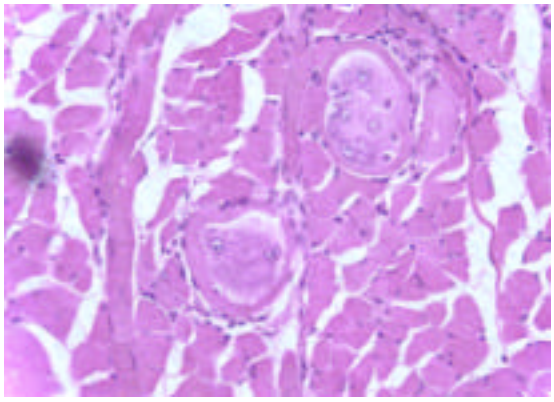
Schistosoma mansonii eggs

Schistosomaisis is a snail-borne disease caused by the flatworm Schistosoma mansonii. It is one of the most common diseases caused by parasitic worms. It is common in the tropics of Asia, Africa, the Caribbean and South America. Almost half a million people in the US are estimated to have the disease. The symptoms include liver damage and malnutrition. The worm is 10-20 mm long and lives in the veins of the intestine. Eggs are discharged into the intestine and carried out of the body in feces. They hatch into a larval form that invades snails, which then release thousands of the second larval form. It is this second larval form that invades humans while they are swimming or wading. Schistosoma mansonii is related to the organisms that cause swimmers' itch.



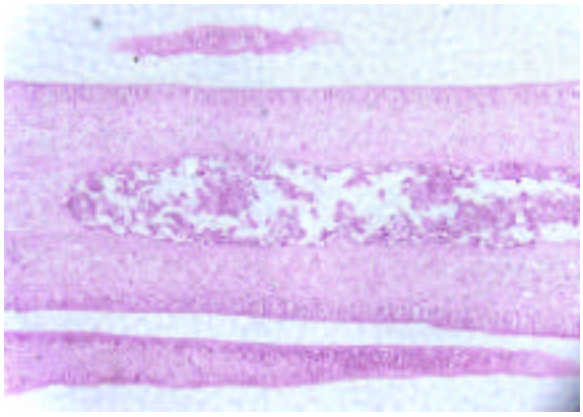
Trichinella spiralis larvae

Trichinella causes trichinosis, a disease mostly found in the northern temperate regions. It is a roundworm (1-4 mm long) that lives in the small intestine of meat eating mammals. It is especially prevalent in wild pigs, rats, and bears. Since the female discharges live offspring directly into the bloodstream, the young can lodge in muscles. Commercially produced pork is only rarely contaminatedwith ***Trichinella***, and bear meat is the major source of trichinosis in North America. If humans eat meat that is infected with ***Trichinella***, it can lead to fever, severe muscle pain, and occasionally death. There is no cure.



Taenia pisiformis

Taenia is the genus of tapeworms. Tapeworms are usually ingested in the cyst form, and the worm emerges from the cyst after passage through the stomach. ***This is a cross section through a portion of the tapeworm.***



Algae
There are a large number of types of algae, and we will be considering only the green algae (Chlorophyta). They can be unicellular, colonial, filamentous, or sheetlike, among others. Only one alga, the green alga ***Prototheca miriformis***, causes human or animal disease. It may be significant that ***Prototheca*** is also unable to make chlorophyll and lives as a heterotroph.

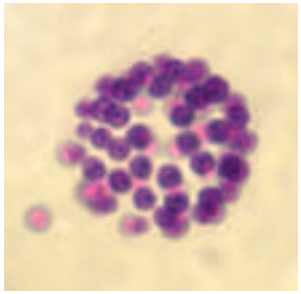
Be able to identify the chloroplasts, nuclei and any unique features of the following algae.

Chlamydomonas (flagellar stain)
Chlamydomonas is a unicellular green alga that has two equal-length flagella, a single nucleus and a single chloroplast, and an eyespot.

Chlorella
Chlorella resembles ***Chlamydomonas***, except that it is nonmotile. It has been used as a source of food. The dried cells are rich in proteins and vitamins.

Gonium
Gonium and ***Eudorina*** are simple colonial algae with only a few cells, and the individual cells resemble ***Chlamydomonas***.

Eudorina

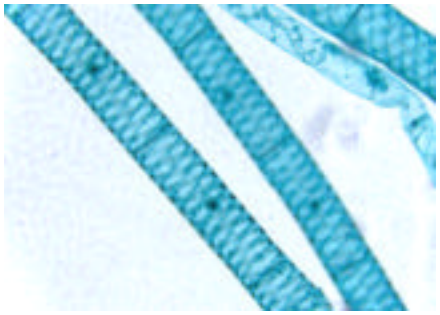


Volvox

Volvox is a large colonial alga that has thousands of ***Chlamydomonas***-like cells in a single layer on the outside. These ***Volvox*** colonies have daughter colonies, each consisting of hundreds of cells, in the hollow inside of the mother colony. Evolutionary biologists think that this series of algae may represent an evolutionary progression from single-celled algae to complex colonial forms.

Spirogyra

Spirogyra is perhaps one of the most recognizable of the filamentous green algae. It has prominent spiral chlorplasts that circle the filament just inside the plasma membrane. Spirogyra is a common component of pond scum. It grows particularly well in water that is nutrient-rich, and is used as an indicator of pollution.



Ulothrix

Ulothrix is another easily recognizable filamentous alga. The filaments are made of short cells, each with a single nucleus and a single chloroplast in a band around the middle. Unlike ***Spirogyra***, it does not grow well in nutrient-rich water, but is instead an indicator of clean water.

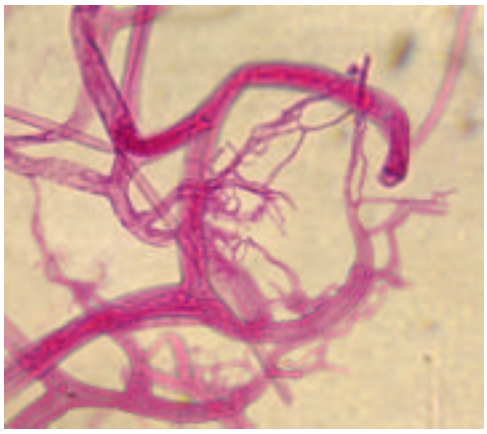
Fungi

Fungi are a diverse group of eukaryotes. They obtain their energy by digesting complex molecules outside the cell and absorbing the simpler molecules through their cell membranes. Most fungi have cell walls, and most have at least a small amount of a polymer called chitin (poly-N-acetyl glucosamine). Molds are fungi that have long, threadlike chains of cells called hyphae (singular: hypha). They often form resistant asexual reproductive cells called conidia. The conidia are frequently formed at the ends of specialized structures called conidiophores, and the shape of these can be used to help classify the fungus. Many fungi can undergo sexual reproduction, and form specialized structures in which haploid sexual spores are formed. Yeasts are single-celled fungi that usually reproduce by budding. Some can reproduce sexually as well as asexually. Some fungi can grow as either a yeast or a mold, depending on the environmental situation, and are called dimorphic.

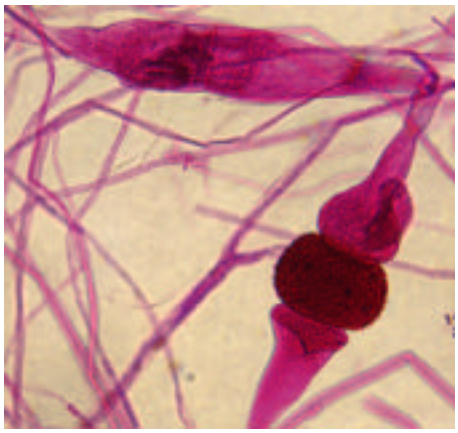
For each of the following fungi, learn to identify hyphae, conidia, conidiophores, sexual spores, yeasts, and molds. Also, learn the differences between ascomycetes, basidiomycetes, deuteromycetes, and zygomycetes, which are four of the largest groups of fungi.

Zygomycetes

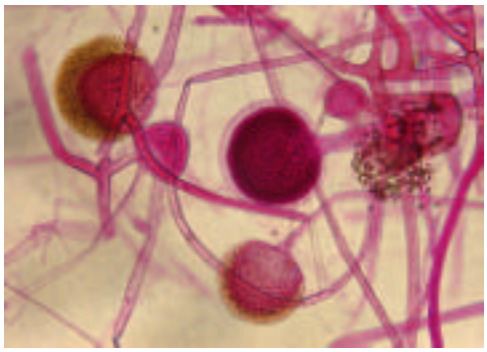
Rhizopus



These are examples of the hyphae of a typical zygomycete. Each hypha has many nuclei, but only rarely have septa dividing the hypha into cells.



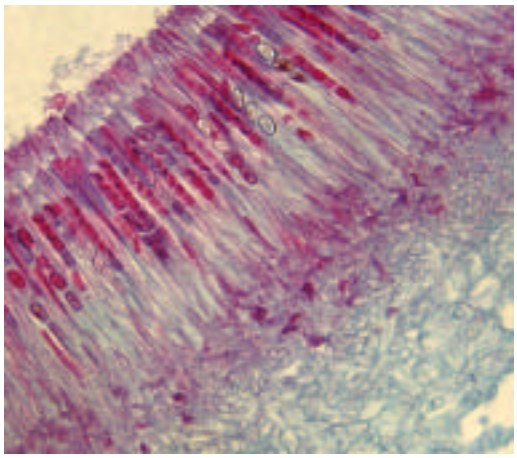
Zygomycetes usually have two sexes, called mating types. When two compatible mating types come in to contact, they will mate and form a large sexual spore called a zygospore. This picture shows an immature spore (top) and a mature one (dark cell at right).



Zygomycetes can reproduce asexually by forming asexual spores inside the round sporangia you see here.

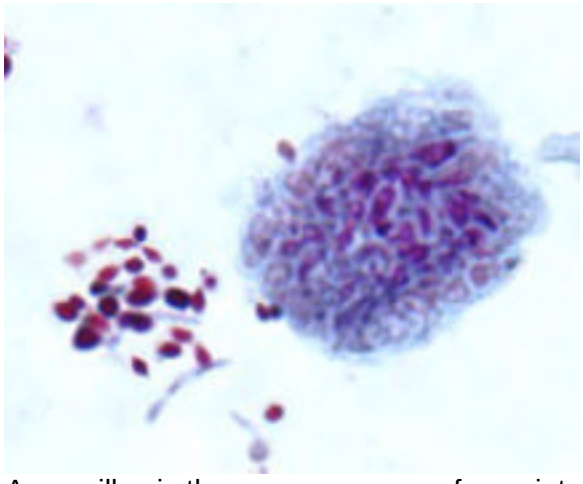
Ascomycetes

Pezizia apothecium



Ascomycetes form sexual spores inside asci (singular: ascus), or sacks that hold spores. Peziza is a typical ascomycete cup fungus in which the inside of the cup is lined with asci that contain football-shaped ascospores.

Aspergillus cleistothecium



Aspergillus is the common name of a variety of cells. This shows the sexual reproductive structure (the cleistothecium) and a group of dark sexual spores (ascospores).

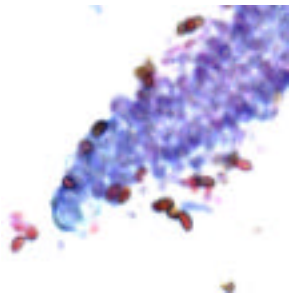
Saccharomyces cerevisiae

Schizosaccharomyces octosporus

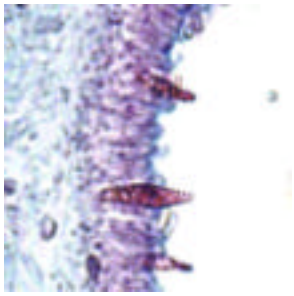
Basidiomcyetes

The most visible members of this group of fungi are the mushrooms and toadstools. These filamentous fungi will mate and send up a mushroom (a fruiting body) in which nuclear fusion and meiosis occur. These slides are sections through the gills of two mushrooms; the darker elongated cells are the spores, and the remainder is the gill material.

Coprinus



Boletus edulis

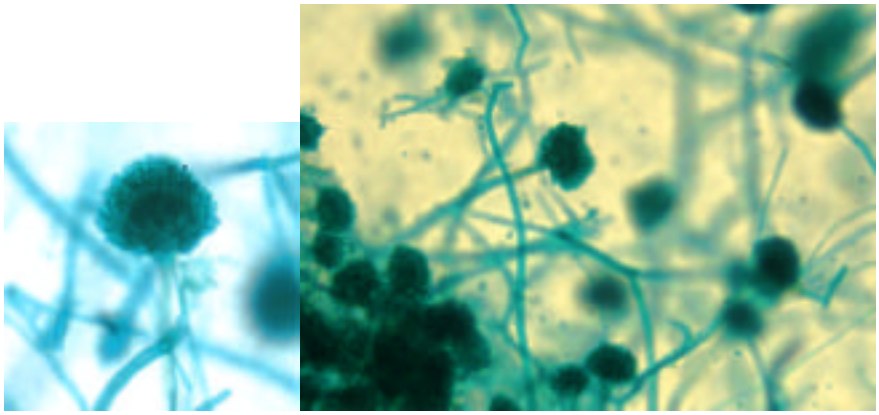


Deuteromycetes

Candida albicans

Candida is a representative of the dimorphic, pathogenic fungi. It can grow as a round, budding yeast, or a filamentous mold. It is a cause of most "yeast infections", including thrush and vaginal yeast infections, and can also cause infections of the skin, nails, and, in immunosuppressed patients, the internal organs.

Aspergillus



Aspergillus gets its name from the asexual reproductive structure that resembles the aspergillum used in some sprinkling rites. The conidia are formed on the outside of a large swollen cell. (Note that these differ from the sporangia of zygomycetes in that in zygomycetes, the conidia are formed internally.)

Penicillium

Penicillium forms its conida on the end of broom-like conidiophores. ***Penicillium notatum*** and ***Penicillium chrysogenum*** are well known because they are the source of the original penicillins.

Bacteria

Eubacteria

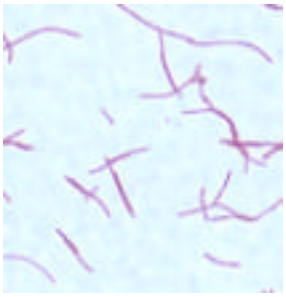
The eubacteria, are one of the three Domains of living organisms (the Archaea and the Eukaryotes are the other two). Only a small fraction of the eubacteria is of medical or economic importance, but those that are can have far reaching effects. Most bacteria are too small to be seen unless you use the 100x objective of the microscope. These are some of the more important, common bacteria. As you observe these specimens, you should learn to identify the various shapes and groupings of the bacteria (bacilli, spirilla, cocci, and chains, pairs, tetrads, and clusters). You should also learn to identify some of the major structures (spores, capsules, flagella). Be aware that not all bacteria have any of these structures.

Clostridium tetani or ***Clostridium botulinum***

Clostridium is the genus of Gram-positive, spore-forming anaerobes. ***Clostridium*** cannot grow in the presence of oxygen. ***Clostridium*** also forms endospores, which are specialized structures that are resistant to many physical and chemical conditions, including heat, dryness, cold, solvents, radiation, and many others. ***C. tetani*** causes tetanus, and it produces a toxin that interferes with relaxation of muscle cells. ***C. botulinum*** causes botulism, and produces a toxin that interferes with nerve transmission.

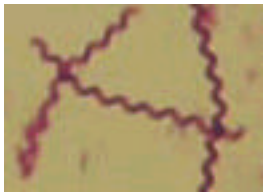
Bacillus anthracis

This is the genus of obligately aerobic, spore-forming, Gram-positive rods. Only one species, ***B. anthracis***, is pathogenic for humans, although ***B. cereus*** can cause a food poisoning. ***Bacillus*** tend to be large: 4.5 -10 µm long by 1-1.25 µm wide. Most strains are motile. As in ***Clostridium***, spores are visible as unstained, refractile inclusions in the cells, or as refractile, free spheres. Spores can be stained using special techniques.



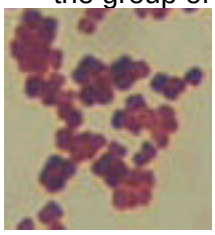
Spirillum serpens

These corkscrew-shaped bacteria are commonly found in water. They are an excellent example of the group of bacteria called spirilla.



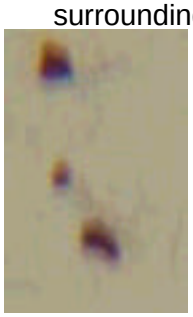
Micrococcus luteus

Micrococcus are (relatively) large spherical cells that grow in clusters. They are examples of the group of bacteria called cocci.



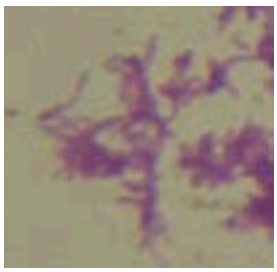
Proteus vulgaris (flagellar stain)

Proteus is covered with many flagella, which allow the bacterium to swim. This preparation has been specially stained to show the tiny, hairlike flagella, which appear to be squiggles surrounding the bacteria. You must view this with 100x magnification.

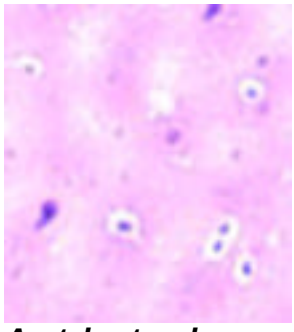


Mycobacterium tuberculosis (acid-fast stain)

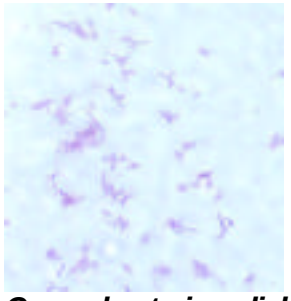
These are long rods 2-5 µm in length, and 0.3-1 µm in diameter. They may occur singly, or in small groups, or occasionally in compact masses. In tissue, they are usually seen as rods, but they can form branching filaments. They cannot be stained by usual methods, because their cell walls contain a waxy material that is relatively impermeable to dyes. They may be stained by the acid-fast method. Robert Koch's early work was on this organism, for which he won the Nobel Prize in Physiology or Medicine in 1905.



Klebsiella pneumoniae (capsule stain)
This small, Gram-negative coccobacilli usually have a very large capsule, composed of polysaccharide, that surrounds each cell.They are typically about 0.5-1.5 µm by 1-2 µm. They are nonmotile. ***K. pneumoniae*** can cause a number of illnesses, including pneumonia, sinusitis, and meningitis. It may carry plasmids that confer multiple drug resistances. This is an example of a capsule stain: The background of the slide is pink, the cells have been stained purple, and the clear area around the cells is the capsule.



Azotobacter chroococcum
This is a large, egg-shaped pleiomorphic rod. It is Gram-variable, and does not make spores, although it does make resistant structures called cysts. ***Azotobacter*** can fix N_2 to NH_3 and amino acids, which they can secrete into the environment. This makes them important in enriching soils.

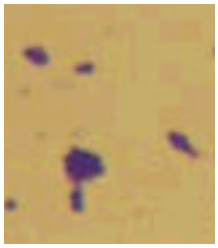


Corynebacterium diphtheriae
This slender rod is about 1-5 µm long and 0.3-0.8 µm wide. The cells tend to be club-shaped, but are very pleiomorphic. They tend to stain irregularly, because they contain storage products that take up dye differently than the cytoplasm does. Some cells may appear to be stained solidly, others are banded. The stained inclusion bodies are called metachromatic granules. It is Gram positive, but tends to destain easily. It can be isolated from the throat of healthy people, but some strains carry a plasmid that encodes the diphtheria toxin. These strains cause diphtheria.

Staphylococcus aureus

This Gram-positive organism grows in clusters that resemble bunches of grapes (***staphyli*** in Greek). There are benign and pathogenic strains of ***S. aureus***. The benign strains can be isolated as normal flora of the human skin and nasopharynx. Pathogenic, invasive strains of S. aureus can be dangerous, and cause boils, carbuncles, and abcesses.

Some strains contain a plasmid that encodes a toxin that causes food poisoning. ***S. aureus*** can often multiply and spread rapidly in tissues becauses it produces extracellular enzymes such as coagulase, and occasionally, toxins. Most strains of ***S. aureus*** carry plasmids that confer multiple drug resistances. It is among the most common causes of hospital-acquired infections.



Streptococcus pyogenes

These spherical Gram-positive organisms grow in chains. Some strains are part of the normal human flora, others cause diseases including impetigo, strep throat, and bacterial endocarditis. Group A streptococci produce more than 20 extracellular substances that are involved in pathogenicity, including hemolysins that lyse blood cells and hyaluronidase that breaks up connective tissue. Particularly aggressive strains are sometimes called "flesh-eating bacteria".

Treponema pallidum

Treponema is the cause of syphilis, an historically important and relatively common sexually-transmitted disease. It is an example of the bacteria known as spirochetes -- very thin and flexible, and is usually not visible with standard microscopy techniques. These preparations have been specially stained to make the treponema visible with light microscopy.

Borrellia burgdorferi

This spirochete is the cause of Lyme disease, one of the emerging diseases found in North America. Normally, it has a transmission cycle that includes the white-tailed deer, the deer tick, and the white-footed mouse. The deer tick transfers the ***Borellia*** between the deer and the mouse, and back again. People can become infected when they become the host for the deer tick. This usually happens when people invade the normal deer/mouse habitat, either for recreation or, as originally happened, when housing developments are built in deer habitat. The bite of the tick usually leads to a diagnostic “bulls-eye” lesion, which can be confused with a rash by people who are less observant.

Neisseria gonorrhea (smear in pus) Obtain from the instructor's table.

There are only a few species in the genus ***Neisseria***. Two of them, N. gonorrhea and N. meningitidis cause disease in humans. N. gonorrhea is transmitted most frequently as an STD, and when it causes symptoms, causes a discharge of pus from the genitals. Untreated, it can lead to scarring of the urethra, vas deferens, and fallopian tubes. Severe cases can result in sterility. If it leaves the reproductive system, it can settle in the joints and cause arthritis. It can also be transmitted vertically from mother to child during childbirth, and can cause gonococcal opthalmia, or an eye infection. All forms of the disease are treatable with antibiotics.

Corynebacterium diphtheriae

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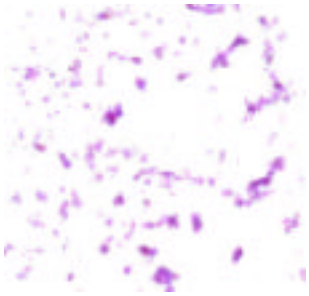


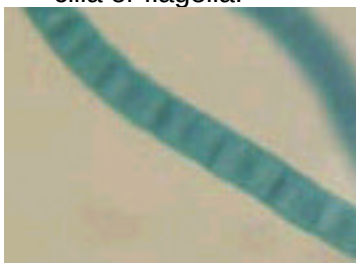
Cyanobacteria

Anabaena
This photosynthetic bacteria has the ability to make everything it needs given water, carbon dioxide, atmospheric nitrogen, and sunlight. Like all cyanobacteria, it carries out photosynthesis in the same way green plants do, with the evolution of oxygen. ***Anabaena*** consists of chains of green vegetative cells with interspersed thick-walled heterocysts. It is within the heterocysts that nitrogen fixation occurs.

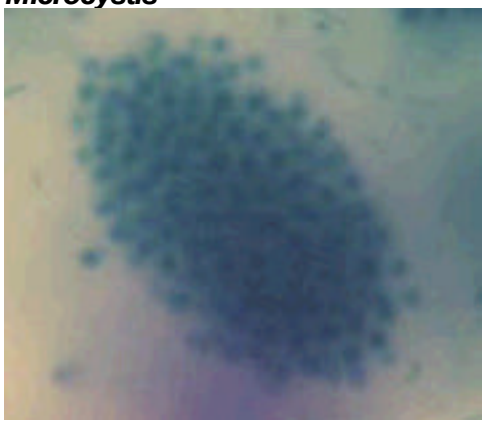
The large round cells in Anabaena and Nostoc are heterocysts: cells that have been specialized to exclude oxygen and allow the enzyme nitrogenase to convert N_2 to NH_3.

Nostoc
This relative of ***Anabaena*** has many of the same characteristics as ***Anabaena***. The major difference is that ***Nostoc*** secretes a gelatinous sheath that encloses many filaments. Single colonies may grow to be 1~12 cm in diameter.

Oscillatoria
This common cyanobacterium is an indicator of polluted water it grows well in nutrient-rich water. The organism makes long filaments that move by a gliding mechanism -- they have no cilia or flagella.



Microcystis



Spirulina