Ubiquity of Microbes

By Krista Granieri (adapted for COA)

Microbes are defined as organisms that are microscopic. In other words, if you need a microscope to see it, it can be called a microbe. In some cases, they are pathogenic, but not always. Many microbes have beneficial relationships with their hosts and some are entirely free-living. Microbes can be found in several kingdoms and include **bacteria, archaea, protists, fungi** and some **helminthic parasites**.

Bacteria and **Archaea** are single-celled prokaryotic organisms. They have no organelles and no nucleus. They reproduce asexually by **binary fission**, which means they simply split in two. Their chromosome (DNA) is usually circular. In addition to their circular chromosomes, they have small extra-chromosomal circular pieces of DNA called **plasmids**. Plasmids typically carry genes for antibiotic resistance, toxin production, metabolic enhancements or structural components such as flagella. Although, bacteria and archaea appear to be structurally simple, they have extremely complex metabolic functions and can survive in an extraordinary variety of habitats. Bacteria are found in our environments and include those that are pathogenic as well as free-living. Archaea are **extremophiles** and live in environments in which most other organisms could not survive, such as underwater volcanic vents, polar ice caps and the Great Salt Lake.

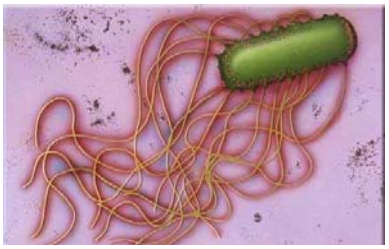


Figure 1: E. coli with sex pili

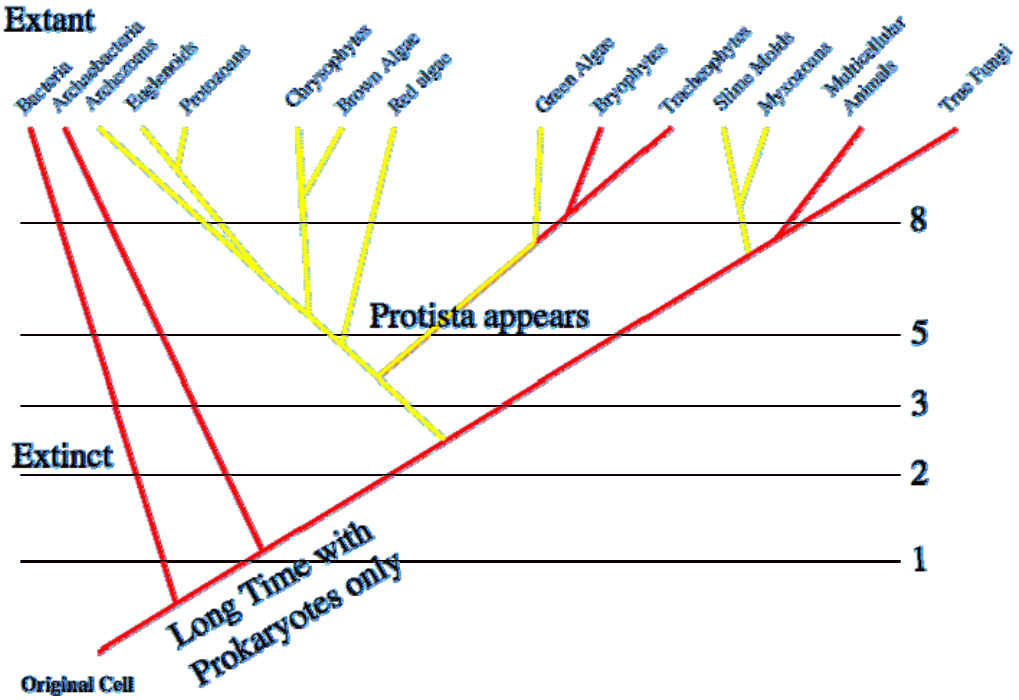


Figure 2: Cladogram of the Extant Kingdoms http://plantphys.info/organismal/lechtml/protista.html

Protists are eukaryotic organisms, which means that they have organelles and a nucleus. They are mostly single-celled, but some species live in colonies and some even live in what could be considered multicellular structures. They have linear chromosomes and also sometimes carry plasmids. They reproduce asexually, by mitosis, and also sexually, by creating gametes. They occupy a large variety of habitats and can be **photosynthetic** or **heterotrophic**. They can be parasitic or free-living. The protists are such a diverse group that many microbiologists think they should be split into more than one kingdom. The protists are almost certainly the ancestors of the three multicellular kingdoms. They can be roughly divided into three groups: animal-like protists, plant-like protists and fungi-like protists.

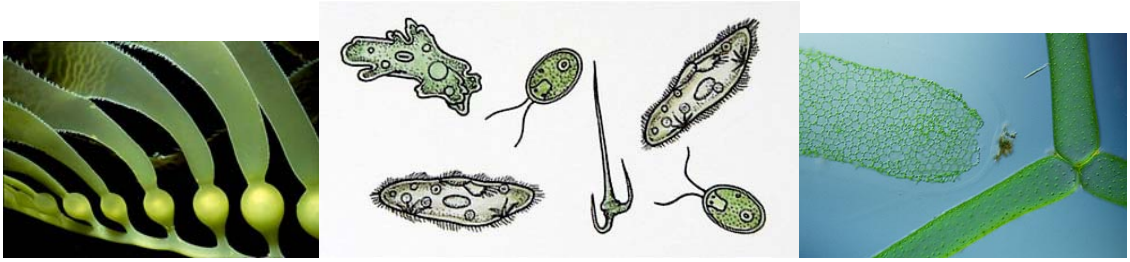


Figure 3:Brown algae, protozoans and green algae (protists)

Fungi are single-celled or multi-celled eukaryotic organisms that are heterotrophic and **saprotrophic** (they decompose dead matter). They come in two basic varieties, **mold** and **yeast.** Molds are multicellular and are composed of structures called **mycelium,** which are formed by **hyphae**. They reproduce sexually and have specialized reproductive structures called **fruiting bodies**. Yeast are single-celled and reproduce asexually by a process called **budding** (it's a lot like mitosis). Some species of fungi can exist as either mold or yeast and are called **dimorphic** ("two forms"), but most exist as one or the other. Another interesting group of "fungi" are the lichens. Lichens are actually a symbiont formed by fungi and green algae. The fungal hyphae surround the algae cells and the two organisms are completely dependant on eachother. The algae can photosynthesize and so it provides sugars for the fungi. The fungi provides protection, moisture and inorganic nutrients to the algae.

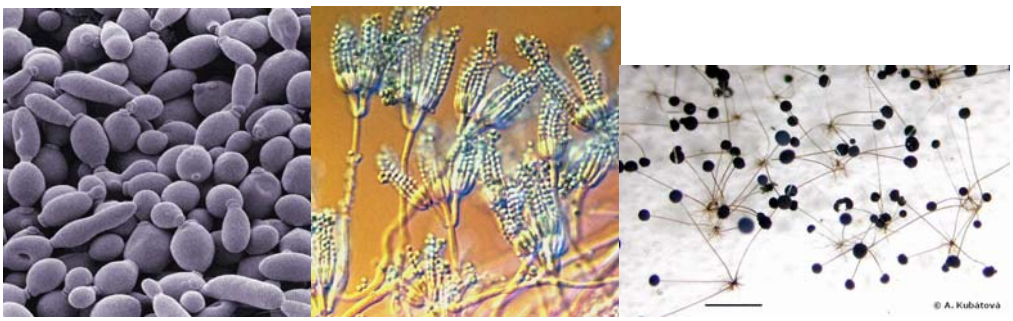


Figure 4: Yeast, Penicillium, Bread mold and Mushrooms (fungi)

Figure 5: Mushrooms (fungi) and a lichen

The **helminthic parasites** are animals in the phylum **nematoda** (non-segmented roundworms) and **platyhelminthes** (flatworms). They are often included in a discussion of microbes since they are microscopic for a significant portion of their life cycle and because they are pathogenic. They tend to be very species specific. That means that they are very picky about what type of host they can live in. Every species of animal has it's own group of parasites. In fact, most biologists believe that there are more species of parasites than free-living organisms in the world.

Figure 6: Tapeworm, fluke and roundworm

In today's lab, we will investigate the morphology and characteristics of each of these groups. We will be using microscopes, so review your microscope lab if you are rusty on how to focus the scope and alter the light settings.

BACTERIA

Quick facts:

- unicellular and prokaryotic
- wide variety of metabolisms (e.g. photosynthetic, heterotrophic, anaerobic, etc.)
- cell walls of peptidoglycan
- reproduce by binary fission

Appearance of Bacteria:

Each species of bacteria has a characteristic cell shape. Bacterial cells also have a way of arranging themselves that is characteristic to each species. Below is a description of the three most common shapes of bacterial cells and the most common arrangements as well.

SHAPES

COCCUS – spherical-shaped cells (plural is cocci)
BACILLUS – rod-shaped cells (plural is bacilli)
SPIRILLUM – twisted rod-shaped cells (plural is spirilli)

ARRANGEMENTS

SINGLE – no particular arrangement (all shapes)
DIPLO – two cells stuck together (can be coccus or bacillus)
STREPTO – cells arranged in a chain (can be coccus or bacillus)
STAPHYLO – cells arranged in clusters (only cocci do this)
Bacteria also have a characteristic way of forming a **colony** that can sometimes help to identify them without the use a microscope. A bacterial colony is a huge mound of cells visible to the naked eye and growing on some kind a **substrate**. Most of the time when we see this it is in some forgotten container of sour cream in the fridge. In the lab, we grow bacteria in **petri plates** on a substrate called **nutrient agar**. **Colony characteristics** include things like **color, shape, texture** and **size**.

Activity 1: Taking Environmental Samples and Observing Colony Characteristics

Week before "microbes" lab:
(Work in groups of 4)

Materials:

- Four (4) petri plates of nutrient agar
- A sharpie
- Several sterile swabs

Methods:

1. Label your plates as shown in the diagram. Draw a line across the bottom of **three** of the petri plates with the sharpie. The bottom is the side with the nutrient agar in it. The empty side is the lid. Be sure you put your name (or initials), the date and the sample course on your plates.

2. Conduct the following environmental sampling:

- Clean/Dirty Fingers
- Body swab (2 places)
- Surface swab (2 surfaces)
- Air monitoring

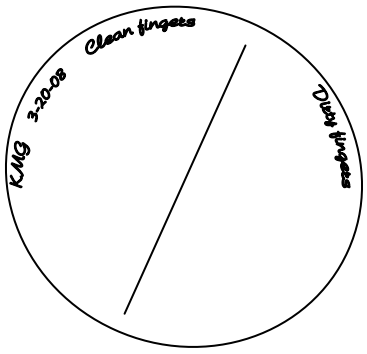


Plate 1: On one side touch the plate with your fingers. Go wash your hands really well (30 secs-SCRUB!!) with soap and warm water. Don't touch anything else after you wash your hands, except for a paper towel. Then come back and touch the other side of the

plate with your clean fingers. Be sure to correctly label which side is “clean fingers” and which side is “dirty fingers”.

Plate 2: Inoculate your second plate using the sterile swabs provided. Select a two places on your body to collect samples and inoculate each side of the plate with a streak (I’ll demonstrate this). Use a new sterile swab for each body swab.

Plate 3: Inoculate your third plate in the same manner as the second plate except, select a surface indoors or outdoors and collect a samples to inoculate each side of the plate with a streak. Use a new sterile swab for each surface swab.

Plate 4: Select a location indoors or outdoors and place you plate there with the lid off for 15 minutes.

Week of the “microbes” lab:

Obtain your plates from last week and look at what has grown on them. Draw diagrams of your plates. Use the dissecting scope to observe as many different types of colony characteristics as you can including color, size, shape and texture if the various colonies.

Activity 2: Observing Bacterial Cells with a Microscope

Observe a prepared slide of three different shapes of bacterial cells and draw an example of each in your lab. You will have to use the highest power objective lens (100X) with immersion oil. You should get your slide into focus at 400X and then call your instructor to help you with the immersion oil. *(Instructors note: If the lab does have enough microscopes with 100X lenses, this can be set up as demo.)*

Activity 3: Stain Your Own Plaque

Bacteria often live in communities called **biofilms**. Biofilms are created because some bacteria secrete a sticky sugary substance called **glycocalyx (gly-ko-kay-lix)**. Glycocalyx makes the bacteria stick to surfaces and it makes other bacteria stick to them. It also serves as food for some of the bacteria in the community. One very common biofilm is the **plaque** on your teeth. You can view the various bacteria in your plaque by taking a sample and applying a stain.

Materials:

- Toothpick
- Clean microscope slide
- Physiological saline dropper bottle
- Methylene blue stain dropper bottle
- DI water in a squeeze bottle
- Several paper towels

Procedure:

1. Place a paper towel on your lab bench and place the slide on it. Place a drop or two of saline on the slide.

2. Use the toothpick to obtain some plaque from your gum line. Gently scrape just under your gums- Be careful! Do not scrape hard enough to draw blood.

3. Dip the toothpick into the saline drops to release some of the plaque onto the slide. The fluid should be just barely cloudy. Smear the fluid with the toothpick into a square the size of a postage stamp and then set it aside to air dry.

4. When the smear has dried completely, place it onto the hot plate on the back counter and let it sit for 2-3 minutes. This “heat-fix” makes the smear stick to the slide better. Use a clothespin to retrieve your slide…IT WILL BE HOT! Place your slide on the paper towel on your lab bench and let it cool until you can handle it.

5. Once the slide has cooled, flood the slide with stain and let it sit for 1 minute.

6. After 1 minute, rinse the slide with DI water. Hold the slide at an angle over the sink and squeeze the water stream against the slide above the smear- not directly at the smear. Rinse until the water runs off pretty clear. You should still be able to see a little blue where the smear is.

7. BLOT- DO NOT RUB- your slide dry. Place the slide on your lab bench on top of several layers of folded paper towels. Place several more layers on top of the slide. Place your hand over the towels and gently press down—Be careful, do not press hard enough to break the slide.

Congratulations! Your slide is now ready to view with the microscope!

8. Observe your plaque at the highest possible magnification and sketch a bit of what you see.

PROTISTS

Quick facts:

- Unicellular, multicellular and eukaryotic
- wide variety of metabolisms (e.g. photosynthetic, heterotrophic, etc.)
- wide variety of ecologies (e.g. parasitic, free living, etc.)
- reproduce by sexually or asexually
- likely ancestors to animals, plants and fungi
- most do not have cell walls

Groups of Protists:

PLANT-LIKE PROTISTS: green algae, red algae, brown algae
ANIMAL-LIKE PROTISTS: flagellates, ciliates, amoebae
FUNGI-LIKE PROTISTS: water molds, slime molds

Activity 4: Observing Protists

Observe the prepared slides of various protists and sketch a few examples. Observe the macroscopic samples of multicellular protists and sketch what you see.

FUNGI

Quick facts:

- Unicellular, multicellular and eukaryotic
- Heterotrophic and saprophytic
- reproduce by sexually and asexually
- cell walls of chitin

Groups of Fungi:

ASCOMYCOTA – yeasts, cup fungi, penicillium
ZYGOMYCOTA – bread molds
BASIDIOMYCOTA – mushrooms

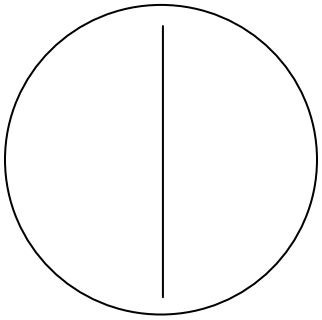
LICHENS – symbionts of fungi and algae

Activity 5: Observing Fungi

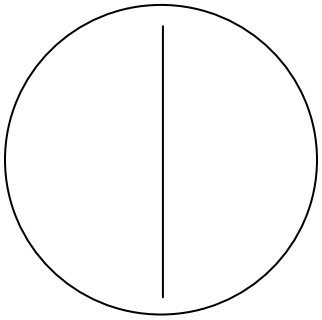
Observe the prepared slides of various fungi and sketch a few examples. Observe the macroscopic samples of fungi and lichens and sketch what you see.

Activity 1: Taking Environmental Samples and Observing Colony Characteristics

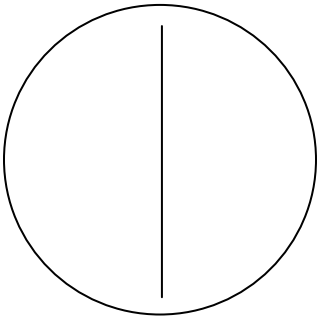
Collect your Petri plates from last week and observe the growth on each one. Pay attention to colony characteristics such color, size, texture and shape. Use the colored pencils to sketch what appears on each plate.



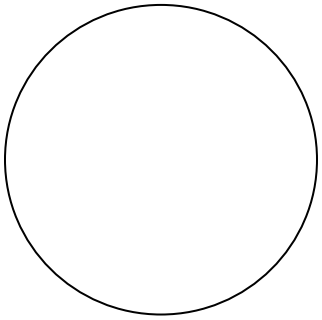
Clean/Dirty Fingers



Body Swabs



Surface Swabs



Air Monitoring

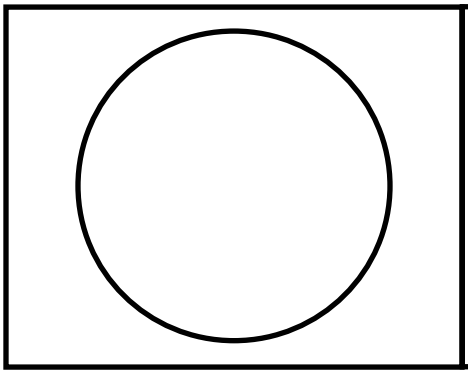
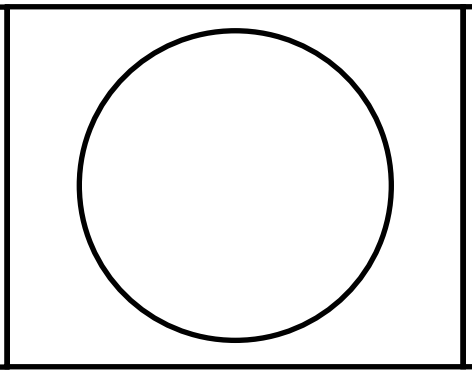
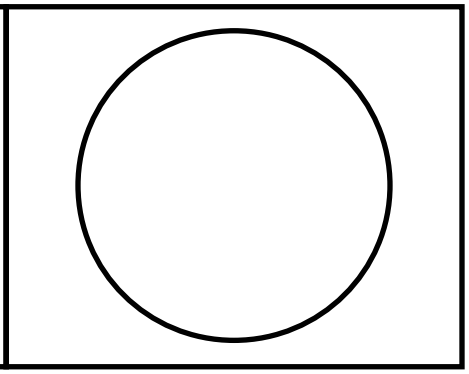
Questions:

1. Which sample had the most growth? Is this what you expected? Explain.

2. Which sample had the least growth? Is this what you expected? Explain.

Activity 2: Observing Bacterial Cells with a Microscope

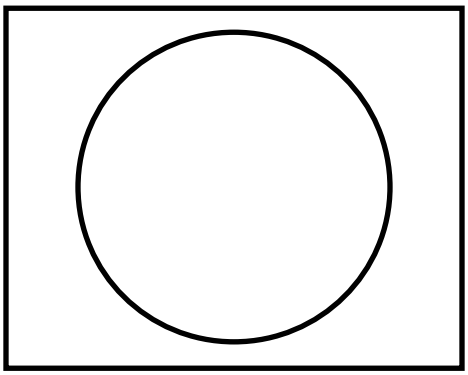
Observe the prepared slides of the three common types (shapes) of bacterial cells. Sketch a few cells from each slide. Be sure to make a note of the magnification used.

COCCI	BACILLI	SPIRAL
		
Total Mag. _____X	Total Mag. _____X	Total Mag. _____X

Activity 3: Stain Your Own Plaque

Sketch a bit of what you see at 100X magnification.

PLAQUE STAIN



Total Mag. _____X

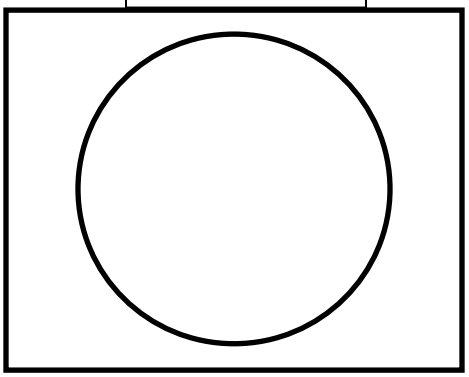
Questions:

1. How many different bacterial species can you find?

2. Are there any non-bacterial cells present? If so, what kind are they?

Activity 4: Observing Protists

AMOEBA



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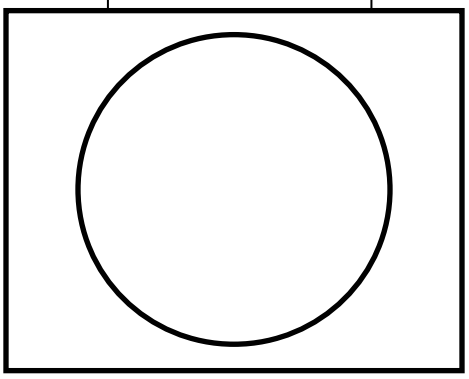
BROWN ALGAE

PARAMECIUM



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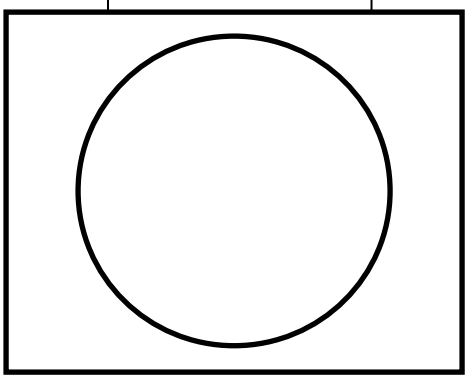
Questions:

1. Are any of these organisms photosynthetic? How can you tell? Which ones?

2. Are any of these organisms multicellular?

Activity 5: Observing Fungi

YEAST



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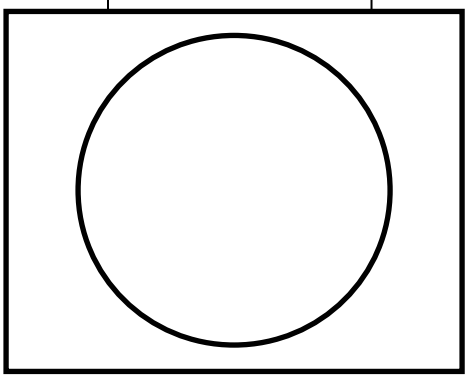
BASIDIOMYCOTA

PENICILLIUM



Total Mag. _____X

LICHEN

Questions:

1. Are any of these organisms photosynthetic? How can you tell?

2. What type of cell walls are present in fungi?