Examination of Natural Microbes

The Antoni van Leeuwenhoek Experiment
A one lab session experiment

Many students taking microbiology for the first time feel that they are thoroughly familiar with the use of the microscope from their previous biology courses. In microbiology, however, you are dealing with such minute forms that it is essential to use good microscope technique. To observe microbes clearly you will especially need proper, focused illumination, a good selection of objectives and patience. In this experiment we will attempt to duplicate the observations Antoni van Leeuwenhoek made with his simple microscopes more than three hundred years ago....that is to observe the major types of microbes: bacteria, algae, fungi and protozoa. We, however, have a definite advantage: the modern microscope. Think about that if you were in fact, Leeuwenhoek, would you have recognized these things as alive?

Materials per pair
1. Pond water with big clumps of algae and other suspended material.
2. Hay Infusion....3 or more days before the lab suspend roughly chopped up grass in a beaker with tap water.
3. Yeast suspension....Make a light suspension of baker’s yeast in tap water just before the lab period.
4. Toothpicks.
5. Pasteur pipettes.
6. Vaseline.
6. Slides and square coverslips.

Procedure

**Pond Water**

1. Take a clean glass slide and place two drops of pond water that contain visible clumps of algae or suspended material. DO NOT USE clear water. Gently place a coverslip over the material and flatten with your finger if necessary. Wipe up any excess liquid with a Kimwipe or towel.

2. Observe first with the 4X lens to center visible clumps and get the microscope in close focus. Switch to the 10X lens and attempt to distinguish the various large forms of microbes: algae and protozoa. When you have located one, switch to the 40X lens and examine in close detail. Measure the cell dimensions with the ocular micrometer and diagram at least two algae and two protozoa.

3. Attempt to discern cell walls, internal organelles such as vacuoles, chloroplasts, nuclei, and flagella if flagellated. Attempt to tell the difference between the cyanobacteria and eukaryotic algae. Diagram any features you can identify.

4. Attempt to see bacteria. Bacteria are the size of mitochondria. You will need to use the fine adjustment: going up and down slowly to see bacteria pop into focus and then out. They should appear clearly outlined when in the plane of focus and dark-blurred as they move out of the plane of focus. You should be able to see rods, cocci and filaments. Which forms are nonmotile? motile? Diagram any bacteria you see.
Hay Infusion

1. Prepare another slide with the hay infusion, but this time make a hanging drop preparation. To do this, build up some Vaseline that will support a cover slip as in the following diagram:

![Diagram of Vaseline and cover slip supporting hanging drop of hay infusion]

2. Place a drop of the hay infusion that is turbid so that the drop hangs cleanly down not touching the slide or the edges of the Vaseline. Observe at low power then move to the high dry lens (40X). Be sure you can distinguish between Brownian motility and true motility.

Yeast Suspension

1. Prepare a standard wet mount of the yeast suspension. Observe first with the 10X lens and then the 40X lens. What sorts of intra-cellular material are evident. What type of cells are yeasts?

Questions

1. What is Brownian motion and why is it generally seen when bacteria are observed in liquid media?
2. How can Brownian motion be distinguished from motility?
3. What are flagella? What is the structural difference between bacterial flagella and eukaryotic flagella?
4. In wet mount preparations, is it possible to see eukaryotic flagella? Bacterial flagella? Why?
5. What advantage does a hanging drop preparation have over a standard wet mount for the observation of motility?
6. What types of major microbes listed in the introduction did you observe for each sample?

Textbook Ref: Brock: Biology of Microorganisms, Section 4.1

See HERE!!! Important!!!

PREPARE for the Next Lab: The Gram stain.

1. Be sure to know about heat fixed smears and the Gram Stain. Read the material in Expt. 2 and in the textbook (section 4.1).

2. Bring in one item from home to Gram stain (hint something with bacteria such as spoiled food, scrapings from your shower curtain, house dust, anything you think has bacteria in it). This will be worth TWO bonus points.

You need to know about heat fixed smears. This will guide your choice of an item to bring! (Bringing in something to Gram Stain is TWO bonus points, students not bringing in something to stain get ZERO, but can use someone else’s material).