# Microbiology Laboratory 3

## Concept - Fungi

Common traits:

- Fungi are *eukaryotic* organisms. As such they have organelles and linear DNA with two copies of each gene thus they are 2n.
- They do not develop tissues but the cells can associate together to form larger *structures* such as mushrooms and hyphae which are long strands of cells attached end to end.
- The cell walls are composed of *chitin* (a polysaccharide).
- Produce *exoenzymes* which degrade complex macromolecules in the environment so the cells can then absorb the smaller molecules as nutrients.
- Fungi can reproduce *asexually* by fission (cell division) or budding.
- Fungi also can produce *spores* for reproduction. The spores can be a part of asexual or sexual reproduction. Much of the traditional divisions are based on the morphological structures of the spores and spore related structures.

Divisions of fungi:

There are four phyla (groups) of fungal organisms you will need to know and you will be required to recognize examples of three of them on an exam. Three of the groups can reproduce sexually.

- ***Rhizopus*** *sp.* - These are the common molds that grow on meat, bread, and cheese. These fungi produce root and stem like hyphae.
  - hint: look for the helmet structure
- ***Aspergillus*** *sp.* - This group includes the edible and poisonous mushrooms. The mycelium (hyphae) are underground but can produce the above ground fruiting body visible as a mushroom. The gills under the mushroom cap hold and release the spores.
  - hint: look for the spiky ball like structure
- ***Penicillium*** *sp.* - This group is also called the "imperfect fungi" because we understand their life cycles imperfectly. The one thing linking the organisms together is that they do not form sexual spores during their life cycle.
  - hint: look for the broom like spore structure
- Ascomycota - These are the "sac fungi and include the yeasts like *Saccharomyces sp.* which are used to make bread, wine and beer. These are the only unicellular fungi, and undergo budding for asexual reproduction. You observed yeast cells in last week's lab.

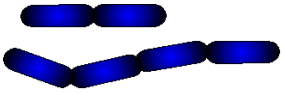
## Lab Assignment

Obtain (and then replace when done) a prepared microscope slide from the front of the room that contains three different kinds of fungal organisms. Observe each kind of fungus on the slide and be prepared to identify each example organism on the next lab quiz. Use the hints above to help in identification.

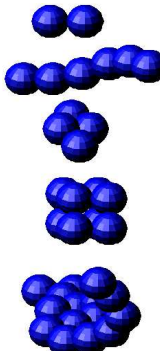
## Concept - Bacterial shapes and arrangements

Shapes - While bacteria can form many shapes, there are three shapes that are most common.
Arrangements - Following division some bacteria stay attached to each other forming various arrangements

- **Bacillus** (bacilli) are rod shaped
  Arrangements include:
  **Diplo**bacilli - appear in pairs after division
  **Strepto**bacilli - appear in chains after division



- **Coccus** (cocci) are round like a ball
  Arrangements include:
  **Diplo**cocci - Cocci that remain in pairs after dividing
  **Strepto**cocci - Cocci that remain in chains after dividing
  **Tetrad** - Cocci that divide in two planes and remain in a group of four
  **Sarcinae** - Cocci that divide in three planes resulting in eight cells together resembling a cube
  **Staphylo**cocci - Cocci that divide in multiple planes to form grape like clusters

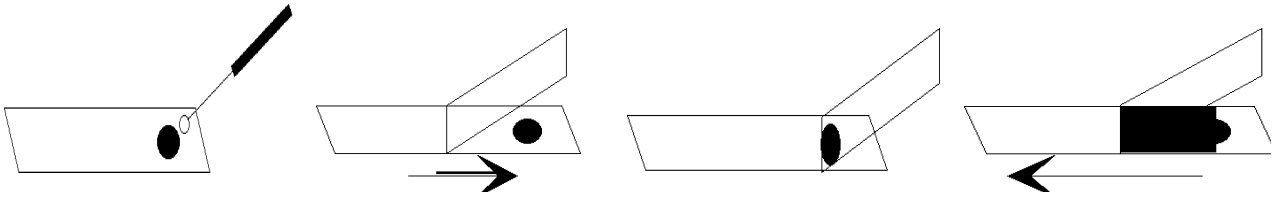


- **Spiral** bacteria have one or more twists
  Arrangements include:
  **Vibrio** - look like curved rods
  **Spirillum** - have a helical shape and fairly rigid bodies
  **Spirochetes** - have a helical shape and flexible bodies

## Negative Stain Technique

The negative stain is great for determining cell size, morphology, and arrangement because the cells are not heat-fixed. The other major use of the negative stain is to observe the presence or absence of a capsule. A capsule, if present, will appear as a halo around the bacteria. The dye used is *nigrosin* which is an acidic stain. This means that the stain readily gives up a hydrogen ion and becomes negatively charged. Since the surface of most bacteria are also negatively charged, the cell surface repels the stain. The glass slide will stain, but the bacteria cells will not. The bacteria show up as clear spots against a dark background.
Procedure

- Place a single drop of nigrosin towards one end of a clean microscope slide.
- Using a flamed loop and sterile technique, transfer some of the cultured organism and mix it into the drop of nigrosin. Be sure there are no large clumps of organism.
- Rest one end of the clean slide on the center of the slide with the stain. Tilt the clean slide toward the drop forming an acute angle and draw that slide toward the drop until it touches the drop and causes it to spread along the edge of the spreader slide. Maintaining a small acute angle between the slides, push the spreader slide toward the clean end of the slide being stained dragging the drop behind the spreader slide and producing a broad, even, thin smear. Allow the film to air dry (this may take some time).
- Make sure there is no stain on the underside of the slide (use a paper towel to dry it if there is). Observe the slide under the microscope. Be sure to look near the edge of the smear! You may need to use oil immersion, as described below.



## Lab Assignment

Make two negative stains to determine the shape and arrangement of your bacteria.

1. Make a negative stain from one of your environmental isolates (from the building in the first lab).

2. Make a negative stain from your organism of the day (given to you by your instructor).

3. View your stains under the microscope. The best areas on your microscope slide to look for bacteria are along the edges where the stain is more gray than black. If you see sharp long white lines you are looking at cracks in the nigrosin stain and not bacteria. You will need to use the oil immersion lens to best observe the bacteria. If you need help, alert your instructor.

4. Put the results of your negative stain of the organism of the day on the blackboard (shape and arrangement (if there is any arrangement)).
   You will want to copy this data down at the end of the lab (most take a picture) to be used in a later lab when identifying your unknown organisms.

## Oil Immersion Technique

- Place the slide in the mechanical stage apparatus. Do not use a coverslip. Put the edge of the smear over the lighted hole in the stage.
- Begin focusing with the 4X (scanning) lens.
- Rotate the nosepiece to view the sample using the 10X (low) objective lens.
- Rotate the nosepiece to view the sample using the 40X (high) objective lens. Use fine focus only to clearly see your subject matter. (Don't forget to change your iris.)
- Turn the nosepiece so that the sample is positioned half-way between the 40X and 100X objective lenses
- Place a drop of immersion oil directly on the sample on the slide.
- Turn the nosepiece until the 100X objective lens snaps into position. Do NOT turn back to the 40X lens! (Don't forget to change your iris.)
- If you look at the lens from the side, it should almost touch the slide and the oil should fill the space between the slide and the lens.
- While looking through the ocular lens, only use the fine adjustment knob to bring the bacteria into focus.
- If the sample is not visible after adjustment of the fine focus, try adjusting the light intensity.
- If this fails, switch back to the 4X (scanning) lens, refocus somewhere else on the slide, then work your way up to the oil immersions lens again.

- When you are finished for the day, be sure to wipe the oil off the oil immersion lens with lens paper before putting your microscope away properly.