



LEVEL:
Year 11&12



TOPIC:
Classification



TIME REQUIREMENT:
45 mins

CURRICULUM ALIGNMENT

- *Biological classification is hierarchical and based on different levels of similarity of physical features, methods of reproduction and molecular sequences (ACSBLO16)*
- *Continuity of life requires the replication of genetic material and its transfer to the next generation through processes including binary fission, mitosis, meiosis and fertilisation (ACSBLO75)*

BACKGROUND

Fungi is a major kingdom among eukaryotic organisms. Fungi do not contain chlorophyll and are heterotrophs, rather than autotrophs. To take in nutrients, Fungi release digestive enzymes into their environment to absorb food. Yeasts are one of the largest groups of fungi. In most cases, the main body of the fungus consists of the mycelium which is composed of hyphae. Reproduction generally occurs through asexual reproduction of spores through mitosis. In cases of sexual reproduction, the spores are produced by meiosis. Before meiosis can occur, two mating strains are often required to cross. The most common dominant phase of the life cycle among fungi is, haploid or $n + n$; unlike the diploid state of most other eukaryotic organisms.

In this practical, students are introduced to the Kingdom Fungi through observation of a few characteristics of a relatively primitive and a highly advanced fungus. To begin exploring Kingdom Fungi, students study two phyla; the zygomycetes and basidiomycetes. Fungi are divided into phyla based on the sexual stage of their life cycles. In Phylum Zygomycota reproduction, the hyphae of the two strains fuse, initiating the sexual phase of the life cycle. The fusion produces a dark zygospore, which constitutes the entire diploid ($2n$) stage of the life cycle. Meiosis within the zygospore restores the haploid state at germination. The zygospore is unique to this phylum. Like Phylum Zygomycota, Phylum Basidiomycota usually reproduces through fusion of + and - strains; however, this occurs before mushrooms form and requires the two strains nuclei to remain separate so that each cell has two nuclei until just prior to spore formation. Furthermore, the nuclei fusion produces a diploid cell (zygote) that undergoes meiosis to produce four haploid spores. This phylum; which includes mushrooms and a number of plant parasites, is characterized by these spores borne on top of a cell or mass of cells called the basidium. This is an excellent opportunity for students to contrast and compare different fungi, as well as gain a closer look at fungi structures and spores under a microscope. Additionally, students learn the differences between sexual and asexual reproduction within the Fungi kingdom.

PREPARATION - BY LAB TECHNICIAN

General Preparations

- 1 If preparing your own media plates, use Potato dextrose agar powder to make the plates at least one day in advance. Keep sealed with a couple of pieces of sticky tape. Store the plates on their lids in the refrigerator.

Preparing Workstations

- 1 Provide each workstation with access to the following materials.
 - Phycomyces live culture, (+) and (-)
 - Inoculating Loops or Wire
 - Bunsen Burner
 - 1 Edible Mushroom
 - Microscope



MATERIALS

- Phycomyces blakesleeenans (Positive Strain)
- Phycomyces blakesleeenans (Negative Strain)
- Edible mushrooms (Supermarket)
- Mushroom Microscope Slide (Entire Pileus)
- Potato Dextrose Agar (or Prepared Plates)
- Inoculating Loops (Disposable)
- Stereo Microscopes



SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment (PPE).
- Know and follow all regulatory guidelines for the disposal of laboratory wastes.
- Dispose of all cultures when you have completed the practical by autoclaving or flooding with disinfectant overnight before proper disposal.
- Take caution when handling fungal cultures and open flames.
- Use sterile equipment and wash your hands before and after the practical.

METHOD - STUDENT PRACTICAL

Creating +ve and -ve Phylum Zygomycota Conjugation Plates

- 1 Draw a line on the base of the petri dish dividing it into 2 halves.
- 2 Mark one side as (+) and the other as (-). Check that the lid is sealed before inverting the plate.
- 3 Determine a place for the +ve that is half way from the central point to the edge of the plate and place the inoculation at this point.
- 4 The easiest way to inoculate a plate is via a straight wire (no loop) that is either kept straight or bent at 90 for the last 3-4 mm.
- 5 Using aseptic technique, pass the sterile, cool, end of the wire through the fungi and then gently scrape the end gently onto the growth agar. This can also be achieved using a plastic disposable loop (no flaming necessary).
- 6 Repeat on the other half of the plate with the -ve strain. The 2 versions will grow and ultimately create a joining line down the centre of the plate.
- 7 Incubate the plate at room temperature for several days until the growth reaches the desired level.
- 8 Once sufficient growth is achieved, open the petri dish and use a stereo microscope or hand lens to observe the fungus and record your observations.
- 9 Observe the plate 3 days after inoculation and record your observations.
- 10 Observe the plate again 7 days after inoculation and record your observations.

Examining Edible Mushroom

- 1 Examine an edible mushroom, note the stalk and cap (pileus).
- 2 Examine the underside of the mushroom cap and locate the gills. The surface of each gill is covered with basidia, each bearing spores. The spores will not be visible with the naked eye due to their small size.
- 3 Remove a section of a gill from a mushroom, make a wet mount slide, and observe the edge of the gill under high power. Try to identify any basidia, spores and hyphae.
- 4 Examine a cross-sectional mushroom slide. Draw and label the structures that you see.

OBSERVATION AND RESULTS

- 3 days after inoculation you should begin to see the beginning of the sexual phase of the life cycle of the phycomyces. This will become evident as the two strains begin to fuse together, to produce a dark zygospore during the second stage of the life cycle. Meiosis occurring within the zygospore will restore the haploid state during germination. Zygospore is unique to this phylum.
- Within 7 days the hyphae (sporangiophores) should grow upward, developing a dark dot at their tip. The dark tips are comprised of asexual spores produced by mitotic divisions, and are known as sporangia. Allowing Phycomyces to reproduce sexually and asexually during its life cycle.

INVESTIGATIONS

- Ask students to identify and describe the hyphae (filaments of cells), and the mycelium. Ask them if they observe any physical differences between the 2 strains of Phycomyces.
- Use charts and prepared slides to show the structure of fungi, including basidia with spores, hyphae, and sporangiophores.
- Contrast how fungi grows in comparison to bacteria. Fungi grow in a different manner to bacteria. From a very small central inoculation on a growth plate, fungi will grow and spread

EXTENSION EXERCISE

Phycomyces is a genus of fungus in the Zygomycota phylum that is known for their strong phototropic response and the helical grow phototropism response of the sporangium. A great extension exercise for this practical, is to allow students to observe phototropic responses in Phycomyces by experimenting with light placement around a sample.