Exercise I

Survey of Higher Microorganisms: Protozoa, Fungi, and Helminths

Kingdom Protista

Protozoa

The Protozoa are a large and diverse group of unicellular, eukaryotic organisms, some of which are parasites. The classification or division into groups is based on the types of locomotion in the mature stage. A typical life cycle includes a vegetative trophozoite and a resting cyst stage.

Phylum Sarcodina or Rhizopoda

This group consists of amoeboid protozoans that use long cytoplasmic processes called pseudopodia ("false feet"). The motility results from the streaming of protoplasm that forms the processes.

Amoeba and Entamoeba are common examples of this group. They feed by taking nutrients into the cell by diffusion and packaging it into food vacuoles. Entamoeba causes amoebiasis or amoebic dysentery, a disease common in areas with poor sanitation. Cysts are ingested in contaminated food and water and develop into trophozoites, which invade the mucosa of the large intestines producing ulcers accompanied by dysentery. Trophozoites can be found in erythrocytes and feces of an infected person.

Phylum Ciliophora

Locomotion is carried out by means of short hair-like projections called cilia. Their synchronous beating propels the organism.

Balantidium coli is the causative agent of balantidiasis. Balantidium exists in two forms: a vegetative trophozoite and a cyst. They are characterized by having two nuclei: a large macronucleus for growth and metabolism and a small micronucleus for cell division.

The cyst form is ingested in contaminated food and water and develops into trophozoites in the large intestine. The large intestines become ulcerated and periods of alternating diarrhea and constipation result.
Phylum Mastigophora

Locomotion in this group is accomplished by one or more long whip-like, thin structures called flagella.

Trypanosoma cause African Sleeping Sickness and American trypanosomiasis. They live in the bloodstream and progressive symptoms include headaches, fever, and anemia. Pronounced fatigue is experienced and most of the time is spent sleeping until death.

Phylum Sporozoa

Unlike other members of Protozoa, Sporozoa do not have structures for locomotion in their mature stages, however, immature forms have some type of movement. All the members of this group are parasites.

The most significant is the genus Plasmodium that causes malaria. They are sporozoan parasites with a complex life cycle, part of which is in various vertebrate tissues, while the other part involves parasites. The sporozoites of the organisms are transferred to a human by the bite of a female Anopheles mosquito. The sporozoites enter the bloodstream and migrate to the liver, where they enter the liver cells and transform into merozoites. Merozoites are released from liver cells and infect erythrocytes, where they undergo asexual reproduction involving sexual stages. The first is called the ring stage, and the second involves the development of schizonts. When mature, the schizont contains a number of merozoites and they are released by lysis from the erythrocytes. The lysis of the schizonts and erythrocytes at once produce symptoms of malaria: chills, which is followed by fever, headache, sweat and malaise. Released merozoites infect new erythrocytes and repeat the cycle. The cycle of attack is 48 to 72 hours depending on the species.

The sexual phase of the life cycle begins when the merozoites enter the erythrocytes and differentiate into male and female gametocytes. When ingested by the female mosquito during a blood meal, fertilization occurs and the zygote develops into a cyst within the mosquito’s gut wall. After many divisions, the cyst releases, sporozoites, some of which enter the mosquito’s salivary glands ready to be transmitted back to the human.

Kingdom Fungi

The fungi include a variety of general forms – yeast, mold, mushrooms, and imperfect fungi. They reproduce in a variety of ways – by a bud arising from a parent yeast cell, by the addition of a vegetative cell to chains of cells called hyphae, and by the production of both asexual and sexual spores. Although fungi form both asexual and sexual spores, they are classified based on the type of sexual spore they form.
Division Zygomycota

Zygomycetes produce sexual spores called zygospores, which are formed from the fusion of hyphae of two different mating types.

Some species of Rhizopus are responsible for producing zygomycosis. Inhalation of spores leads to invasion of the blood and eventually necrosis of tissues, especially in diabetic and immunocompromised individuals.

A single mating type of Rhizopus produces dark-colored structures called sporangia. Each sporangium contains many asexual sporangiospores. Rhizopus is a common bread mold, its hyphae are haploid and non-septate (coenocytic), and cytoplasmic streaming within them is common. Surface hyphae called stolons are anchored by rhizoids where the hyphae contact surfaces. Asexual sporangiospores are produced by sporangia at the end of elevated stalks called sporangiophores. These spores develop into hyphae identical to those that produced them.

Sexual reproduction occurs when hyphae of different mating types (+ and - strains) make contact. Progametangia extend from each hyphae and upon contact a septum separates the end of each progametangium into a gamete. The walls between the two gametangia dissolve, the gametes fuse and a thick-walled zygospore develops. The zygospore germinates and produces a sporangium. Haploid spores are released that develop into new hyphae, and the life cycle is completed.

Division Ascomycota

Fungi in this division form sexual spores called ascospores within a saclike structure called an ascus. Aspergillus and Penicillium are filamentous members of this group, whereas the budding yeast Saccharomyces is a unicellular member. A bud is an outgrowth from the parent cell that pinches off, producing a daughter cell.

Aspergillus species are opportunistic. One form of pulmonary aspergillosis (fungal balls) involves colonization of the bronchial tree or tissues damaged by tuberculosis. Allergenic aspergillosis occurs in individuals who are in frequent contact with the spores and become sensitized to them. Subsequent contact produces symptoms similar to asthma. Invasive aspergillosis is the most severe form and results in necrotizing pneumonia and may spread to other organs.

The Aspergillus fruiting body is distinctive, with chains of conidia containing asexual conidiospores arising from a swollen vesicle at the end of a stalk called a conidiophore.

Penicillium is best known for its production of the antibiotic penicillin. Asexual reproduction occurs with a fruiting body, which consists of conidia containing spherical conidiospores in chains at the end of elongated branching cells.
Division Deuteromycota

Candida albicans is a deuteromycete that is part of the normal microbiota of the skin, in the mouth, and in the vagina. It reproduces asexually by conidospores on a long chain of cells.

Kingdom Animalia

Helminths (worms)

The worms that are parasitic to humans and other animals are collectively referred to as helminths. Helminths include the flatworms of the Phylum Platyhelminthes, which are subdivided into flukes (Class Trematoda) and tapeworms (Class Cestoda). Helminths also include roundworms (Phylum Nematelminthes, Class Nematoda).

Phylum Platyhelminthes

Class Trematoda

Flukes are non-segmented and are usually grouped according to the site of problems – blood, liver, lung, and intestinal. They are flattened dorsoventrally, and have an oral sucker for feeding and a ventral sucker for attachment.

Clonorchis sinensis, the Chinese liver fluke, begins its infestation when larva called metacercariae, are ingested in raw or undercooked freshwater fish. Once ingested, the metacercariae move to the bile ducts where they become adults. They are monoecious so a single fluke can produce fertilized eggs that each contains a viable larval form called miracidia. The eggs leave the body in feces and enter fresh water. A snail ingests the miracidia which undergoes asexual multiplication in the snail and emerges as cercariae. The cercariae encyst in fish muscle tissue and become metacercariae.

Class Cestoda

Adult tapeworms live in the small intestines, where they attach by a scolex or head that has hooks and up to four suckers. The neck, or growing region, is directly behind the scolex and is followed by segments called the immature proglottids. Mature proglottids containing the reproductive organs follow the immature proglottids. The gravid proglottids are the oldest and they each contain a uterus filled with eggs.
Phylum Nemathelminthes

Class Nematoda

Roundworms are cylindrical, have non-segmented bodies, and are tapered at each end. The body is covered in a tough cuticle that protects it from the gastric juices and enzymes of digestion. Undercooked pork containing encysted larva of Trichinella spiralis is the means by which humans become infected. Humans are infected by eating insufficiently-cooked pork or bear. The meat contains encysted larva that are freed by gastric digestion. The larva pass into the duodenum, where they develop into mature male and female worms. After mating, the female burrows into the mucosa of the intestinal villi, where she enters the lymphatic system and deposits fully developed, viable larva in the lymph nodes to be carried through the body by the bloodstream where they ultimately encyst in skeletal muscle tissue. The severity of trichinosis symptoms depends on the number of viable larva ingested. There may be gastrointestinal discomfort as the intestinal mucosa is invaded. Larva migration is accompanied by muscle pain, chills, weakness, and occasionally prostration. Respiratory distress and myocardial involvement result if the larva encyst in the diaphragm or heart muscle, and death can occur.

Procedure

Obtain the following slides and locate the structures on the diagrams:

Amoeba
Entamoeba
Balantidium
Trypanosoma
Plasmodium
Rhizopus sporangia
Rhizopus zygotes
Aspergillus
Penicillium
Saccharomyces
Candida albicans
Clonorchis
Taenia
Trichinella

Clean-up Procedures

1. Clean the slides used under oil immersion with Kim wipes.
2. Return the slides to the appropriate trays.
3. Clean the objective lenses of the microscope with lens paper and ethyl alcohol.
4. Return the microscope to the cart.
Kingdom Protista

Protozoa

Phylum Sarcodina or Rhizopoda

Amoeba

Entamoeba

Phylum Ciliophora

Balantidium
Phylum Mastigophora

Trypanosoma

Phylum Sporozoa

Plasmodium
Kingdom Fungi

Division Zygomyycota

sporangium
sporangiospores

zygospore

Asexual spores

Sexual spores

Rhizopus

Division Ascomycota

conidiospores

Asexual spores

Aspergillus

Penicillium

Asexual spores
Phylum Cestoda

scolex

hooks

sucker

immature (young) proglottids

matute proglottid

genital pore

testes

ovary

gravid proglottid

Taenia

uterus

uterus with eggs
Phylum Nemathelminthes

Trichinella