

Exercises 11/18/20

Fungus – Exercise 11

43. Differentiate the macroscopic and microscopic appearance of mold versus yeast.

Familiarize with terms:

What is the term for a fungus that is able to grow as yeast under certain conditions and mold under other conditions?

Dimorphic

What is the term for multicellular filaments in mold?

Hyphae

Name for hyphae separated by crosswalls?

Septate hyphae

Name for hyphae not separated by crosswalls?

Aseptate (coenocytic) hyphae

Name for hyphae that grow above the agar surface and produce asexual reproductive spores?

Aerial hyphae

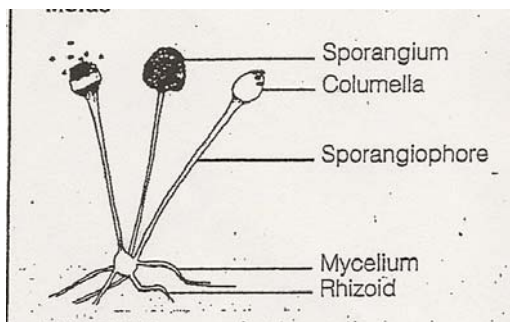
Name for hyphae that grow on or down into the agar surface to extract nutrients from the medium?

Vegetative hyphae

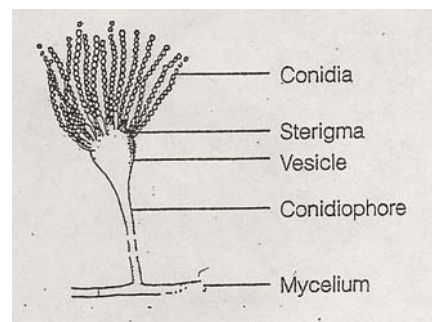
Name the two types of asexual spores.

Sporangiospores: form at end of aerial hypha

Conidia: form on hyphae



Sporangiospores



Conidia

Macroscopic Features

Mold

- Fuzzy or hairy colonies
- Colorful
- Height of aerial hyphae
 - Cottony: very high aerial hyphae
 - Velvety: medium high aerial hyphae
 - Woolly: medium low aerial hyphae
 - Powdery: very low aerial hyphae

Yeast

- Appearance: Moist
- Color: White and cream
- Smells like bread

Microscopic Features

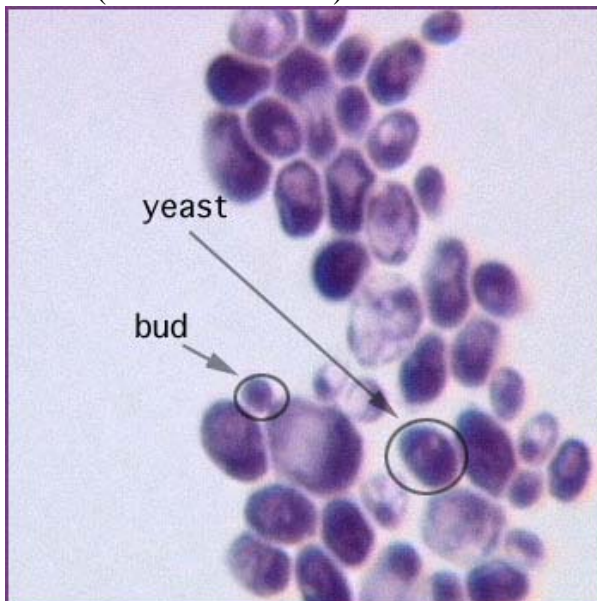
Mold

- Septate or aseptate structure
- Type of asexual sporulation (Sporangiospores or Conidia)

Yeast

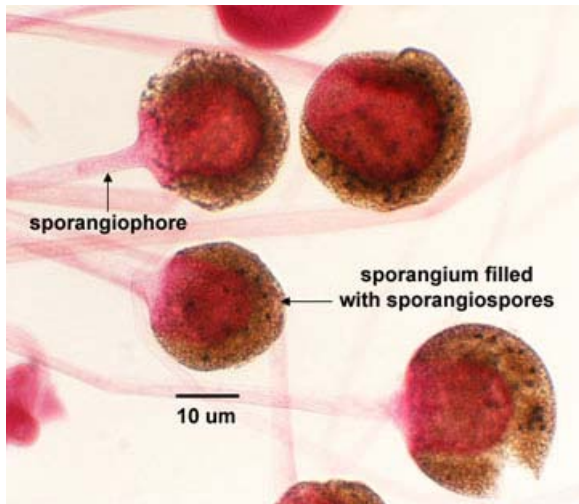
- 5 to 10 times larger than bacteria
- Round to oval in shape
- Reproduce asexually by budding

Yeast: (*Candida albicans*)



Look for: Budding

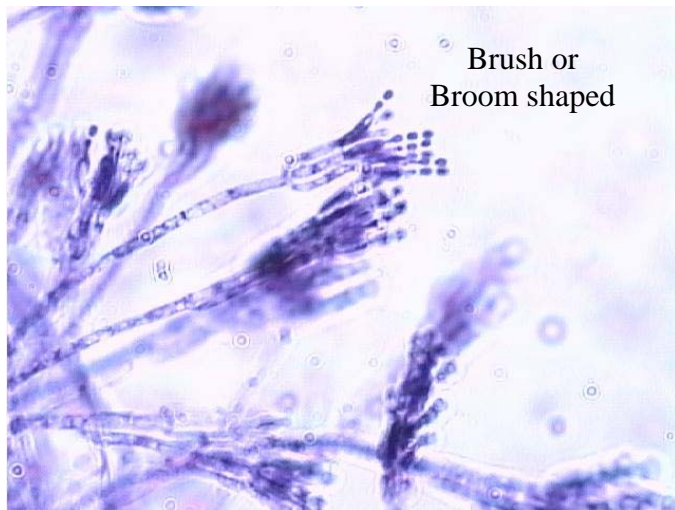
Mold:



Rhizopus



Aspergillus



Penicillium

44. Given a chart of the common mold sporulation patterns, identify an unknown mold based on its macroscopic characteristics and microscopic sporulation.

THE MOST COMMON MOLDS

Adapted from Cappuccino, *Microbiology: A laboratory Manual*, 7th edition, 2005, p.239-241

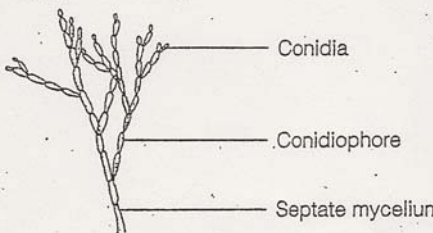
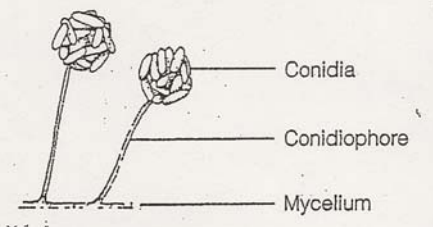
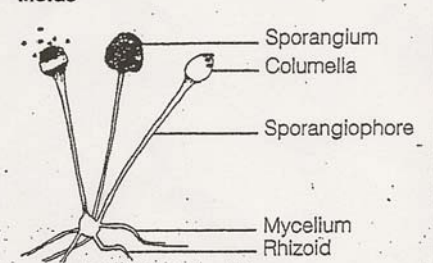
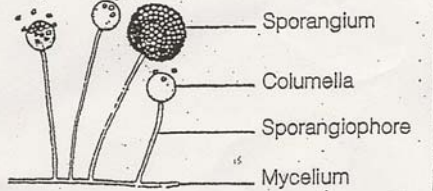
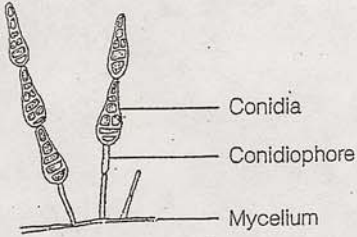
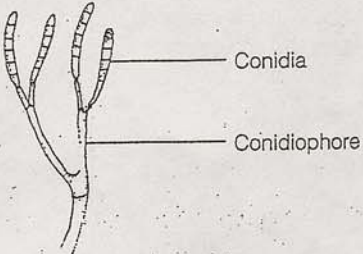
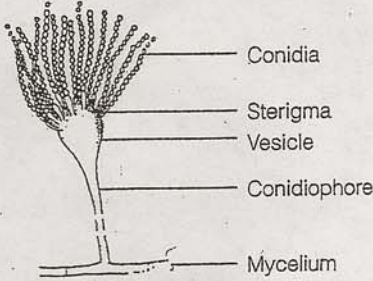
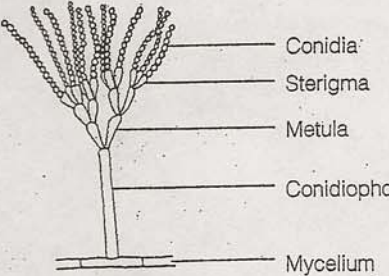
Diagram	Colonial Morphology	Microscopic Appearance
<p>Molds</p>  <p>Conidia</p> <p>Conidiophore</p> <p>Septate mycelium</p> <p><i>CLADOSPORIUM:</i> DEAD AND DECAYING PLANTS</p>	<p>Small, heaped colonies are greenish-black and powdery</p>	<p>Spores (conidia) develop at the end of complex conidiophores arising from a septate mycelium that is usually brownish</p>
 <p>Conidia</p> <p>Conidiophore</p> <p>Mycelium</p> <p><i>CEPHALOSPORIUM:</i> ANTIBIOTIC PRODUCTION</p>	<p>Rapidly growing compact and moist colonies becoming cottony with aerial hyphae that are gray or rose colored</p>	<p>Single-celled conical or elliptical spores (conidia) held together in clusters at the tips of the conidiophores by a mucoid substance; erect, unbranched conidiophores arise from a septate mycelium</p>
<p>Molds</p>  <p>Sporangium</p> <p>Columella</p> <p>Sporangiophore</p> <p>Mycelium</p> <p>Rhizoid</p> <p><i>RHIZOPUS:</i> BLACK BREAD MOLD; COMMON LABORATORY CONTAMINANT</p>	<p>Rapidly growing white-colored fungus swarms over entire plate; aerial mycelium cottony and fuzzy</p>	<p>Spores are oval, colorless, or brown; nonseptate mycelium gives rise to straight sporangiophores that terminate with black sporangium containing a columella; rootlike hyphae (rhizoids) penetrate the medium</p>
 <p>Sporangium</p> <p>Columella</p> <p>Sporangiophore</p> <p>Mycelium</p> <p><i>MUCOR:</i> FOOD CONTAMINANT</p>	<p>Resembles the colonies of <i>Rhizopus</i></p>	<p>Spores are oval; nonseptate mycelium gives rise to single sporangiophores with globular sporangium containing a columella; there are no rhizoids</p>

Diagram	Colonial Morphology	Microscopic Appearance
<p>Molds</p>  <p>Labels: Conidia, Conidiophore, Mycelium</p> <p><i>ALTERNARIA:</i> NORMALLY FOUND ON PLANT MATERIAL</p>	<p>Grayish-green or black colonies with gray edges rapidly swarming over entire plate; aerial mycelium not very dense, appears grayish to white</p>	<p>Multicelled spores (conidia) are pear-shaped and attached to single conidiophores arising from a septate mycelium</p>
 <p>Labels: Conidia, Conidiophore</p> <p><i>FUSARIUM:</i> FOUND IN SOIL</p>	<p>Woolly, white, fuzzy colonies changing color to pink, purple, or yellow</p>	<p>Multicelled spores (conidia) are oval or crescent-shaped and attached to conidiophores arising from a septate mycelium</p>
 <p>Labels: Conidia, Sterigma, Vesicle, Conidiophore, Mycelium</p> <p><i>ASPERGILLUS:</i> PLANT AND ANIMAL PATHOGENS; SOME SPECIES USED INDUSTRIALLY</p>	<p>White colonies become greenish-blue, black, or brown as culture matures</p>	<p>Single-celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophore, the vesicle; long conidiophores arise from a septate mycelium</p>
 <p>Labels: Conidia, Sterigma, Metula, Conidiophore, Mycelium</p> <p><i>PENICILLIUM:</i> ANTIBIOTIC-PRODUCING CITRUS FRUIT CONTAMINANT; SOIL INHABITANT</p>	<p>Mature cultures usually greenish or blue-green</p>	<p>Single-celled spores (conidia) in chains develop at the end of the sterigma arising from the metula of the conidiophore; branching conidiophores arise from a septate mycelium</p>

Microscopic appearance of the most common molds
 from Benson, *Microbiological Applications* 8th edition, 2002, p.53



- (1) *Penicillium*— bluish-green; brush arrangement of phialospores. (12) *Pullularia*— black, shiny, leathery surface; thick-walled; budding spores.
- (2) *Aspergillus*— bluish-green with sulfur-yellow areas on the surface. *Aspergillus niger* is black. (13) *Diplosporium*— buff-colored woolly surface; reverse side has red center surrounded by brown.
- (3) *Verticillium*— pinkish-brown, elliptical microconidia. (14) *Oospora* (*Geotrichum*)— buff-colored surface; hyphae break up into thin-walled rectangular arthrospores.
- (4) *Trichoderma*— green, resemble *Penicillium* macroscopically. (15) *Fusarium*— variants, of yellow, orange, red, and purple colonies; sickle-shaped macroconidia.
- (5) *Gliocadium*— dark-green; conidia (phialospores) borne on phialides, similar to *Penicillium*; grows faster than *Penicillium*. (16) *Trichothecium*— white to pink surface; two-celled conidia.
- (6) *Cladosporium* (*Hormodendrum*)— light green to grayish surface; gray to black back surface; blastoconidia. (17) *Mucor*— a zygomycete; sporangia with a slimy texture; spores with dark pigment.
- (7) *Pleospora*— tan to green surface with brown to black back; ascospores shown are produced in sacs borne within brown, flask-shaped fruiting bodies called pseudothecia. (18) *Rhizopus*— a zygomycete; spores with dark pigment.
- (8) *Scopulariopsis*— light-brown; rough-walled microconidia. (19) *Syncephalastrum*— a zygomycete; sporangiophores bear rod-shaped sporangioles, each containing a row of spherical spores.
- (9) *Paecilomyces*— yellowish-brown, elliptical microconidia. (20) *Nigrospora*— conidia black, globose, one-celled, borne on a flattened, colorless vesicle at the end of a conidiophore.
- (10) *Alternaria*— dark greenish-black surface with gray periphery; black on reverse side; chains of macroconidia. (21) *Montospora*— dark gray center with light gray periphery; yellow-brown conidia.
- (11) *Bipolaris*— black surface with grayish periphery; macroconidia shown.

Viral Plaque Assay: Exercise 18

What is an obligate intracellular parasite?

A parasite that can only grow inside another living host cell (Viruses)

What are bacteriophages (aka phages)?

Viruses that infect bacteria.

What is the word we use to describe viruses that burst open their host cell, killing the cell?

Lytic

Describe a plaque.

A plaque is a clear area on a plate of bacterial growth that indicates where a virus has infected the bacteria and caused them to lyse.

What does PFU stand for?

“Plaque-forming unit”. Each plaque on the Petri plate indicates the presence of a virus particle or PFU.

Why do we make dilutions of the T-4 virus?

We make dilutions in order to obtain countable plates so we can then calculate the titer of phage in the original substance. We must make several dilutions because we don't know which dilution will produce a countable plate. A countable plate for T-4 plaques is 20 to 200 plaques.

Why is T-4 used in the lytic cycle and not in the lysogenic cycle?

Because T-4 can only work in the lytic cycle. T-4 lyses the cells to release phage.

See Appendix C in your lab book for instructions on how to calculate. Calculate dilutions for the following problems (watch your decimal points!):

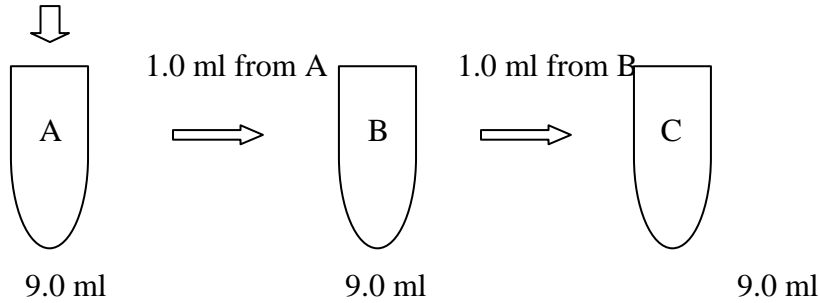
Calculate plaques:

Problem 1:

- One ml of original sample is added to tube A, which has 9 ml of sterile water.
- After mixing, 1 ml from tube A is put into tube B, which contains 9 ml of sterile water.
- After mixing, 1 ml from tube B is added to the 9 ml of sterile water in tube C.

What are the dilutions of the original water sample in tubes A, B & C?

1.0 ml water sample



Tube A	
Tube B	
Tube C	

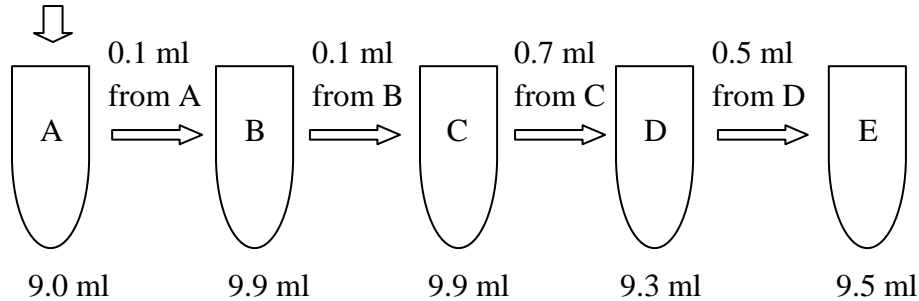
Answers	
Tube A	1:10
Tube B	1:100
Tube C	1:1000

Problem 2:

- One ml of original sample is added to tube A, which has 9 ml of sterile water.
- After mixing, .1 ml from tube A is added to tube B, which has 9.9 ml of sterile water.
- After mixing, .1 ml from tube B is added to tube C, which has 9.9 ml of sterile water.
- After mixing, .7 ml from tube C is added to tube D, which has 9.3 ml of sterile water.
- After mixing, .5 ml from tube D is added to tube E, which has 9.5 ml of sterile water.

What are the dilutions of the original water sample in tubes A, B, C, D & E?

1.0 ml water sample



Tube A	
Tube B	
Tube C	
Tube D	
Tube E	

Answers

Tube A	1:10
Tube B	1:1000
Tube C	1:100,000
Tube D	7:10,000,000
Tube E	7:200,000,000

Calculate the PFU:

Again, read Appendix C in your Lab Manual. A countable T-4 plate, when T-4 virus infects E. coli, has 20 to 200 colonies, or plaques. Calculate the titer of phage (recorded as PFU per ml) in the original sample for each of the following tables.

PFU counted x Dilution of Plate = PFU per ml

Table 1

Plate	PFU Counted	Dilution of Plate
1	500	1:10
2	52	1:100
3	6	1:1,000

Which Plate is countable: _____ PFU per ml

Answers: Use plate #2: $52 \times 100 = 5,200$ PFU per ml

Table 2

Plate	PFU Counted	Dilution of Plate
1	189	1:10
2	17	1:1,000
3	0	1:1,000,000

Which Plate is countable: _____ PFU per ml

Answers: Use plate #1: $189 \times 10 = 1,890$ PFU per ml

Acquiring Bioluminescence by Transformation: Exercise 20

45. Describe the genes located on the plasmid used in this exercise.

What does GFP mean?

Green fluorescent protein (it shines under UV light; also codes for GFP)

What is the name of the plasmid used in this exercise?

Plasmid GLO (pGLO) (GFP is put into pGLO through genetic engineering)

What gene confers specific antibiotic resistance to bacteria by expressing the protein “penicillinase” (beta-lactamase)?

Ampicillin resistance gene (AMP)

What is the gene that codes for the regulator (regulates transcription of GFP gene)?

Ara C

How can the Ara C regulator be blocked?

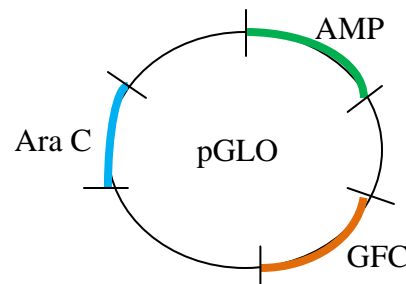
By adding Arabinose to the media.

What three genes are located on the pGLO plasmid?

Ampicillin resistance gene (AMP)

Green fluorescent protein (GFP)

Ara C



46. Define “competent” and describe two methods used to make the *E. coli* competent?

What are cells that are able to act as the recipient in transformation?

Competent cells

Are most cells competent?

No

What are the two methods of making cells competent?

- Shocking the bacterium with cold/hot temperatures
- Exposing the bacterium to magnesium chloride (MgCl) or calcium chloride (CaCl)

47. Determine if the E. coli on an agar has been transformed.



Plate 1

Results of the exercise:

Plate #	Growth	Glow
Plate 1: pGLO on Luria agar + Ampicillin + L-Arabinose (Transformed: produced penicillinase)	Growth	Glow
Plate 2: pGLO on Luria agar + Ampicillin	Growth	None
Plate 3: Control on Luria agar	Growth	None
Plate 4: Control on Luria agar + Ampicillin	No growth	None

48. Explain the purpose of the Luria broth, arabinose and ampicillin in this experiment.

What is the purpose of the Luria broth in this experiment?

The Luria broth is a media used for bacteria growth. It contains nutrients for growth and gene expression. E. coli will grow, replicate, and have protein synthesis (penicillinase & GFP).

What is another name for gene expression?

Protein synthesis

What is in the Luria broth?

Carbohydrates, amino acids, nucleotides, salt (NaCl) & vitamins

Why is Arabinose use in this experiment?

Arabinose blocks the regulate (Ara C) to allow translation and transcription of GFP.

Why is Ampicillin used in this experiment?

Ampicillin is an antibiotic used to stop growth. As seen in Plate 3 (Control), E. coli is allowed to grow. In Plate 4 (Control), Ampicillin is present and there is no growth.