EVOLVING YEASTS – WEEK 1

EXPERIMENT 1. Seed your yeast in their new growth media.

1. Take two two tubes filled with growth medium, one labelled “10 μM Fungicure” and one labelled “No Fungicure”.
2. Label both tubes with your group name and date.
3. Using a sterile swab, dip it into the yeast culture that you have been provided with.
4. Transfer the damp swab (the liquid in the cotton bud will have millions of yeasts stuck to it) to the tube labelled “No Fungicure”.
5. Mix the growth media in the new tube with the swab – remove the swab and dispose of in a pot of 10% bleach.
6. Using another sterile swab, dip it into the yeast culture that you have been provided with.
7. Transfer the damp swab to the tube labelled “10 μM Fungicure”.
8. Mix the growth media in the new tube with the swab – remove the swab and dispose of in a pot of 10% bleach.

Now, look at the yeast cultures. Take a picture, describe what the yeast culture looks like. Is it see-through or opaque (cloudy/muddy)? Compare the tubes that you have seeded to the tubes that you were given. **Record all of your observations in your lab book** (print out your pictures and add them to your book, if possible).

Place both tubes in the “Growth rack”.

Allow the yeast to grow in the tube for **one week** in a rack at room temperature (feel free to swirl the media every day or so – this will help the yeasts grow).

For next week please watch this video and make some notes:

Online tutorial for using a micropipette:
https://www.youtube.com/watch?v=uEy_NGDfo_8

EVOLVING YEASTS – WEEKS 2-14

It has now been one week since the yeasts were placed in the growth medium. Retrieve your tubes from the "Growth rack".

Gently swirl the tubes to suspend the yeasts.

Now, look at the yeast culture. Again, take a picture, describe what the yeast culture looks like. Is it see-through or cloudy? Look at the photo from last week – notice any changes? How does the Fungicure™ treated sample look compared to the non-Fungicure™ treated? **Record all of your observations in your lab book** (print out your pictures and add them to your book if possible).

1. **IMPORTANT:** If the growth of the yeast **with Fungicure™** looks to have improved compared to previous weeks and the non-treated yeast, then double the concentration of Fungicure™ in the growth medium. If not, use the same concentration as previous weeks (we start at 10 μM final concentration by adding 20 μL from stock tube number 1).

EXPERIMENT 2. Now to seed your "No Fungicure" yeast in their new growth media.

1. Label a tube with group name, date and "No Fungicure™".
2. Using a sterile swab, dip it into the yeast culture labelled "No Fungicure" that you grew **last week**.
3. Transfer the damp swab (the liquid in the cotton bud will have millions of yeasts stuck to it) to the new culture labelled "No Fungicure" that you have prepared in step 1.
4. Mix the growth media in the new tube with the swab – remove the swab and dispose of in a pot of 10% bleach.
5. Place the tube in the "Growth Rack" and allow to grow for 1 week at room temperature.
6. Place last week's culture in the "Settling Rack"

EXPERIMENT 3. Follow these steps to prepare your Fungicure™ growth media for seeding with yeast:

Online tutorial for using a micropipette:
https://www.youtube.com/watch?v=uEy_NGDfo_8

1. Consult table 1 to decide which concentration of Fungicure™ you should be adding to your 2 mL of growth media.
2. Label each tube – group name, date and concentration of Fungicure™.

3. Take a pipettor and ensure that the number reads "020" on the side, this means 20 µL.
4. Push a sterile plastic tip onto the end of the pipettor.
5. Push down the plunger to the "first stop".
6. Dip the tip into the desired tube of Fungicure™ that you need.
7. **Gently** release the plunger and suck up the Fungicure™ into the plastic tip.
8. To add the Fungicure™ into you culture tube, remove the lid of your culture tube, place the plastic tip onto the tube, and gently press down on the plunger.
9. Replace the cap and swirl the media to mix.

Table 1. Determining the amount of Fungicure™ to add to your yeast cultures

Final concentration of Fungicure™	Stock tube of Fungicure™	Volume to add to your yeast growth medium
10 µM (starting concentration)	**1**	**20 µL**
20 µM	2	20 µL
40 µM	4	20 µL
80 µM	8	20 µL
160 µM	16	20 µL

EXPERIMENT 5. Now to seed your yeast in their new growth media.

1. Using a sterile swab, dip it into the yeast culture **with** Fungicure™ that you grew **last week**.
2. Transfer the damp swab (the liquid in the cotton bud will have millions of yeasts stuck to it) to the new culture labelled "10, 20, 40, 80, or 160 µM Fungicure" that you have prepared in EXPERIMENT 3.
3. Mix the growth media in the new tube with the swab – remove the swab and dispose of in a pot of 10% bleach.
4. Place the tube in the "Growth Rack" and allow to grow for 1 week at room temperature.
5. Place last week's culture in the "Settling Rack"

Allow the yeast to grow in the tube for **one week** in a rack at room temperature (feel free to swirl the media every day or so – this will help the yeasts grow). **REPEAT** this process every week during this semester to see if we can evolve Fungicure™ resistance in the classroom!

SETTLING and STORING YEAST

This will allow samples to be returned to UI for future genetic analysis.

- Old cultures should be allowed to settle out for one week in the “settling rack” so that they can be prepared for storage.

- After the yeasts have been settling for 1 week - upturn tubes over a pot of 10% bleach to remove the growth media and keep the yeast. Yeast should stick to the bottom of tube.

- Place the tubes in the “Storage Rack” in the refrigerator.

SAFETY NOTES

CONTAMINATION: If you notice that the color of your yeast culture has changed or that the culture has gone moldy – **DO NOT OPEN the tube**. The tube has become contaminated and the entire tube (including the liquid) should be immersed in 10% bleach. Tubes should be left for 20 minutes before disposal of the liquid down the sink and tubes into the trashcan.

DISINFECTION: Anything that has touched the yeast culture (should only be the sterile swabs) should be immersed in 10% bleach and left for 20 minutes before disposal of the liquid down the sink and solids into the trashcan.

SPILLS: If there are any spills of the yeast cultures, they should be blotted with paper towels by placing the towels over the spill. Then the towels should be sprayed with 10% bleach and left for 10 minutes before cleaning up.

If you spill yeasts on your hands, wash then thoroughly with hot soapy water. If the yeast splash into your eyes, flush them with warm running water. Yeast splashed on clothing should be blotted and washed with soapy water.

FUNGICURE: Fungicure™ solution (1% Clotrimazole) is available commercially over the counter and has been extensively diluted to 0.1% for in class use. The small quantities used in our experiments **do not** pose a significant health hazard. The Fungicure™ is dissolved in 100% isopropanol which is extremely **FLAMMABLE**, keep away from flames and other sources of ignition. Isopropanol may cause skin irritation and severe eye **irritation <wear protective eyewear when pipetting Fungicure™>**. If you spill Fungicure™ on your hands, wash then thoroughly with warm soapy water. If Fungicure™ splashes into your eyes, flush them with warm running water.