Extracting Yeast from Sourdough Starter

Sofia Karti and Alex Broussard, Ph.D.

Stuart Hall High School, San Francisco, CA

Last updated November 2021

OVERVIEW

This experiment aims to isolate yeast from a sourdough starter into individual colonies to produce a pure yeast culture. A sourdough starter is a culture that harbors both lactic acid bacteria and yeast in combination with water and flour. Our main goal is to distinguish between these two organisms, specifically the yeast, and the first three experiments are there to guide you to avoid the bacteria and culture the yeast from the sourdough starter. The product of those experiments will give us slides to look under the microscope, where you will then be able to confirm whether you have successfully cultured yeast. Overall, this experiment will help students understand what a thriving yeast culture looks like to identify them in the future.

MATERIALS AND EQUIPMENT

Below are the following materials that are necessary for performing **Experiments 1-3**:

Microbes
- Small amount of sourdough starter

Equipment
- Incubator set to 30°C
- Light microscope capable of at least 400X magnification
- P10 or P20 micropipette and tips
- 4 glass test tubes, at least 5mL each
- Test tube caps or aluminum foil
- Bunsen burner

Consumables
- 4 YPD agar plates
- YPD growth media
- Inoculation loops
- Microscope slides
- Paper towels

Chemicals
- Methylene Blue

PPE
- Gloves
- Safety glasses or goggles

EXPERIMENT 1. Isolate Single Colonies of Yeast and Bacteria (Day 1 & 2)

Procedure:

1. Wash your hands and put on gloves and safety goggles. (this is true for every experiment unless indicated otherwise)
2. Pick up a small amount of sourdough starter with an inoculation loop.
3. Using the isolation streaking technique, streak three or four dense swipes on the top of the agar plate.
4. Flip the inoculation loop to the dry side and place it on the last place touched by the inoculation loop.
5. Swipe the remaining sourdough starter across the rest of the agar plate.
6. Place the dish in the incubator for 24-48 hours at 30°C.

EXPERIMENT 2. Growth of Yeast Culture (Day 3 & 4)

1. Remove your sourdough dough culture dish from the incubator.
2. Confirm that you can see single colonies at the bottom of the agar plate before removing the lid.
3. If you are unable to see any colonies, redo steps 1-5 from Day 1 & 2 and use less sourdough starter.
4. If you can see colonies, grab a micropipette and remove the lid off the agar plate.
5. Inoculate a YDP liquid culture with your single colony using a sterile pipette tip.
6. Repeat these steps four more times to get four chances of growing a yeast culture.
7. Cover the test tubes with a cap or a small amount of tin foil and wrap around the top of the test tube.
8. Place the four test tubes in the incubator for 24-48 hours at 30°C.

EXPERIMENT 3. Prepare Yeast/Bacteria Cultures to Look Under the Microscope (Day 5)

1. Remove the test tubes from the incubator.
2. Homogenize the culture by pipetting or vigorous shaking.
3. Use a micropipette to extract a drop of liquid (10ul works well) and place it on a microscope slide.
4. Leave the slides out for 15-20 minutes to dry.
5. Once dry, heat the slide using a Bunsen burner. (For this step do not wear gloves, to prevent burning.)
6. Place one drop of methylene blue towards the end of each slide and let them sit for 2-3 minutes.
7. Use a plastic pipette to wash off the excess methylene blue from each slide.
8. Dry the slides carefully using a paper towel.
9. Your slides are now ready to look under the microscope.

Important note: *In an undisturbed culture, yeast cells typically settle to the bottom of the tube by gravity and appear as an off-white sediment. Many bacterial species will produce a uniformly cloudy culture. We recommend checking cultures for turbidity and immediately bleaching turbid cultures.*

EXPERIMENT 4. Identifying if Cultures are Yeast or Bacteria (Day 6)

1. Now that you have your slides ready, you need to look under the microscope to see if you have successfully cultured yeast or cultured bacteriaRemove the test tubes from the incubator.
2. Move the stage of the microscope to its lowest position.
3. Place your slide on the stage.
4. Select the lowest power objective lens (10x).
5. Turn the coarse focus knob until you are able to see cells.
6. Then, select the next highest power objective (40x).
7. Finally, turn the fine focus knob until you can see the cells clearly.
8. If you see that your culture has large circular cells and is most commonly clumped together, you have successfully cultured yeast cells! For reference, an average yeast cell is 4 to 12 microns long. A successful yeast culture is pictured below.

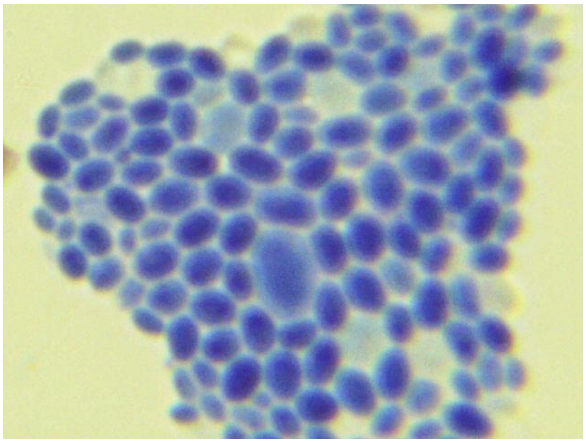

Fig. 1: Yeast culture viewed at 400X

9. If your culture has smaller cells that are not clumped together but instead take up your whole view, you have cultured bacteria. For reference, an average bacterial cell is 0.5-1 micron long. A bacteria culture is pictured below.

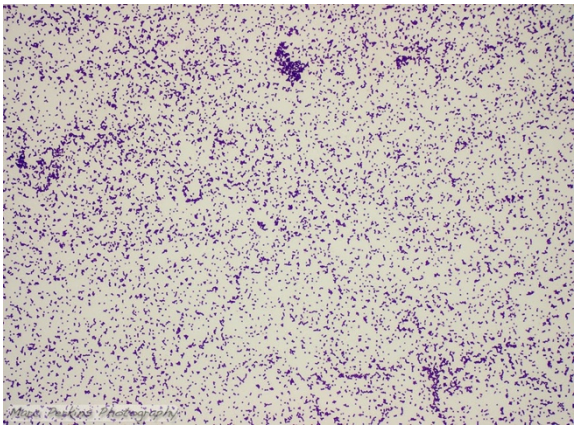

Fig. 2: Bacterial culture viewed at 400X

10. Repeat steps 1-9 for tubes 1-4.