

Teacher Preparation Notes for Is Yeast Alive?

by Dr. Ingrid Waldron and Jennifer Doherty, Department of Biology, University of Pennsylvania, 2008¹

Equipment and Supplies:

Baker's yeast (preferably rapid rising super active; make sure the yeast has not reached its expiration date) (see Teacher Preparations 1, below)
Sugar (see Teacher Preparations 1, below)
Plastic zip-lock baggies (2 per group)
Small water balloons (4 per group) (see Teacher Preparations 1, below)
Test tubes, between 15-25 mL (4 per group) (see Test Tubes or Substitutes, below)
Test tube rack (1 per group)
Container for water that will hold at least 100 mL (1 per group)
Gloves (optional, ~2 per group)
Sharpies (1 per group)
Sterile nutrient agar plate (1 per group) (see Sterile Nutrient Agar Plate Preparation, pages 2-3)
Microscope(s), slides and coverslips (2-4 per group) (see Microscope Supplies, below)

Test Tubes or Substitutes:

You can purchase a variety of plastic test tubes at www.testtubesonline.com for less than 10 cents each (sometimes much less). You can also purchase test tube racks for \$4.35. If you have only very limited budget, supplies, and equipment, you can do the first half of the lab activity (the test for metabolism) using yeast, sugar, small water balloons, and the plastic tubes used to hold single cut flowers or very small bottles which have narrow necks that will fit into the ends of the water balloons. Take care to keep the volume of whatever container you chose small enough so it and the balloons fill up with carbon dioxide within 25 minutes using a reasonable amount of yeast.

Microscope Supplies:

Purchase from Carolina Biological:

632962	22 × 22 mm Coverslips	\$3.55 Box of 100
632950	Microscope slides	\$6.95 Box of 72

If you do not have access to reasonable quality compound microscopes (yeast cells are 5-10 μm in diameter), this lab activity can be done just as well by simply omitting step 6 on page 4 of the student handout.

Teacher Preparations:

1. You will need to experiment with your yeast and size of test tube to determine how much yeast you need for four test tubes. We have found that approximately 1 g of yeast and 1.5-2 g of sugar per 25 mL test tube provide good results. 1 sugar packet is 4.3 g of sugar. For best results, use small water balloons and make sure the seal between the test tube and water balloon is tight. If you use large test tubes (100ml or greater) regular sized balloons work well.

¹ These teacher preparation notes and the related student handout are available at http://serendip.brynmawr.edu/sci_edu/waldron/.

- At least one day before class, prepare one Petri dish of yeast growth medium per group, as described in the following section.
- At the beginning of class, have ready group kits of 4 test tubes, 4 balloons, 1 zip-lock bag with an appropriate amount of yeast and another zip-lock bag with an appropriate amount of sugar, together with a test tube rack, sharpie, and container for the students to get warm water. You may want the students to wear gloves then they shake their test tubes to mix the yeast.
- For experiment 2, have the students use only 10-12 grains of yeast and a small amount of water. If incubating at room temperature allow 3-4 days for growth. If you can incubate at 37° C, then overnight will be sufficient.

Sterile Nutrient Agar Plate Preparation:

There are three ways of obtaining sterile nutrient agar plates. Although options 1 and 2 are more expensive, we recommend them if you do not have experience preparing sterilized media.

- Buy plates that are pre-poured with sterile nutrient agar. About \$1.80 per plate.
821862 Nutrient Agar, Prepared Media Plates 100 x 15 mm, Pack 10 \$17.95
- Buy solid sterile nutrient agar medium that you microwave to liquefy and then pour into sterile Petri dishes. See pouring instructions below. About \$1.30 per plate.
82-1045 Nutrient Agar Media Kit for Preparing 20 plates \$25.95
- Prepare sterile nutrient agar from powder using an autoclave or a stove-top pressure cooker and then pour into sterile Petri dishes. Simply boiling the agar is not sufficient for sterilization and your plates will be contaminated with bacteria. Between \$0.75-0.40 per plate.
789374 Nutrient Agar Dehydrated Media Set for Preparing 40 plates \$30.30
or
173651 Yeast-Extract Dextrose Medium for Preparing 100 plates \$15 or 173650 for Preparing 25 plates \$4.95
741250 100 × 15 mm Petri dishes for 20 plates \$4.95

To do this, add the appropriate amount of nutrient agar and distilled water (see table below) into a flask or glass bottle and cover with aluminum foil. When using an autoclave or pressure cooker always use a container that is twice the volume of the liquid you are sterilizing. To sterilize the solution you want to keep the autoclave or pressure cooker at 15 psi for 20 minutes. To use the pressure cooker, add about 1" of water to the pot, place the covered glass container in the pot, and close and lock the lid. Following the instructions for your pressure cooker, start timing 20 minutes after the pressure cooker has reached the right pressure. After sterilizing, use caution when removing the pressure cooker lid so you do not get scalded with steam. Let the agar cool to 50°C before pouring plates.

Nutrient Agar + Distilled Water = Yield

Nutrient Agar	Distilled Water	Yield
23 g	1000 ml	50 plates
11.5 g	500 ml	25 plates
9.2 g	400 ml	20 plates
4.6 g	200 ml	10 plates

Pouring Plates:

When pouring sterilized media into sterile Petri dishes it is important to always keep the agar covered and the lid on the Petri dish unless you are actively pouring in agar in order to avoid contamination.

1. Pour enough of the sterilized agar medium (cooled to approximately 50°C) into each sterile plastic Petri dish to cover the bottom—about 1/8" to 1/4" deep. You do not need to remove the cover of the plate completely; you can just lift the lid enough to pour in the agar. When you have poured the plate lower the lid immediately. If the medium solidifies before you finish pouring, it can be reheated in the microwave.
2. Place the covered agar plates on a countertop to cool and solidify. Agar medium will set like stiff gelatin at room temperature.
3. The agar medium is now ready for storage or use. **Storage: Do Not Freeze!** Stack agar plates **upside down** in the refrigerator. The purpose of placing the plates upside down is to prevent condensation from dripping down onto the agar surface which could then facilitate movement of organisms between colonies. If plates have been refrigerated, set them out and allow them to warm to room temperature before using them.

Teaching Points

- The characteristics of life include using energy (i.e. metabolism), ability to grow and develop, reproduction, homeostasis, response to the environment, evolutionary adaptation, composed of one or more cells, and has genetic material.
- The first experiment indirectly tests for the ability to metabolize, i.e. utilize energy. When sugar is available, the yeast metabolizes the sugar and produces carbon dioxide, a gas which accumulates in the balloons and causes them to get bigger.
- Replication of each experimental condition is useful to be more confident of your results, since experimental results are often variable even when you try to maintain the same conditions.
- The second experiment tests for the ability to grow.
- Some things that look dead are actually alive in dormant forms that can survive long periods in difficult environments (e.g. too dry or lacking in food), until the environment improves and provide the conditions needed for active metabolism and growth.

Possible Addition to This Activity

If your students can use boiling water, they can design additional experiments to test whether treating the grains of yeast with boiling water kills them and prevents subsequent metabolism and growth. This provides further evidence that the production of gas and growth occurred because the yeast grains were alive. However, this only works if the yeast grains are treated with water which is boiling or very close to boiling and not merely hot.

Related Activities

One related activity, "Cellular Respiration in Yeast", investigates the effects of sugar concentration and other variables on the rate of metabolism in yeast (available on this website). Another activity, "Taste Test: Can microbes tell the difference?", measures the rate of yeast metabolism with different foods such as artificial sweeteners and different beverages (available at <http://www.asm.org/Education/index.asp?bid=35292>). Another activity, "Yeast on the Rise", tests the rate of rising in bread doughs that differ in the concentrations of sugar or other ingredients (available at <http://www.microbeworld.org/resources/experiment/pgs62-65.pdf>).