Yeast Busters: Stopping Fungus in its Tracks with Antifungal Medicines

https://www.sciencebuddies.org/science-fair-projects/project-ideas/BioMed_p012/medical-biotechnology/stopping-fungus-with-antifungal-medicines
(http://www.sciencebuddies.org/science-fair-projects/project-ideas/BioMed_p012/medical-biotechnology/stopping-fungus-with-antifungal-medicines)

Last edit date: 2018-04-26

Experimental Procedure

Working with Biological Agents

For health and safety reasons, science fairs regulate what kinds of biological materials can be used in science fair projects. You should check with your science fair's Scientific Review Committee before starting this experiment to make sure your science fair project complies with all local rules. Many science fairs follow Intel® International Science and Engineering Fair (ISEF) regulations. For more information, visit these Science Buddies pages: Projects Involving Potentially Hazardous Biological Agents (http://www.sciencebuddies.org/science-fair-projects/project_src_biological_agents.shtml) and Scientific Review Committee (http://www.sciencebuddies.org/science-fair-projects/project_src.shtml). You can also visit the webpage ISEF Rules & Guidelines (http://www.societyforscience.org/Page.aspx?pid=312) directly.

Before you start the experiment, read about the antifungal agents you picked to test and find out how they stop fungal infections.

Setting up the Gas Collection Apparatus

In the first part of this procedure, you will be setting up the gas collection apparatus. In the process of growing, yeast produce carbon dioxide (CO_2), and if the number of living yeast decreases, the amount of CO_2 produced will also decrease. Consequently, measuring CO_2 production is a good way to determine how much yeast is alive relative to the other conditions you test. The gas collection apparatus that you set up here will show you how much CO_2 is being produced by the yeast.

1. Remove the small red cap from one of the squeeze bottles. Then connect the tubing to the tip opening, as shown in Figure 2. Make sure that you have a tight fit.

Figure 2. Tube connected to the bottle opening.

2. You will be collecting carbon dioxide from the yeast by displacing water trapped in an inverted graduated cylinder. Here's how to set it up:
    a. Fill your plastic dishpan (or bucket) about one-third full with water.
    b. Fill the 100-mL graduated cylinder with water.
        i. If your dishpan is deep enough, fill the graduated cylinder by tipping it on its side inside the dishpan. Allow any bubbles to escape by tilting the cylinder up slightly, while keeping it under water. Keeping the opening of the cylinder under water, turn it upside down and attach it to the side of the dishpan with packing tape (or have your helper hold it in place).
        ii. If your dishpan is not deep enough, fill the graduated cylinder completely using the faucet and cover the top tightly with plastic wrap. Quickly invert the cylinder and place the opening in the dishpan, beneath the surface of the water. Remove the plastic wrap. Attach the cylinder to the side of the tub with packing tape (or have your helper hold it in place).
    c. The graduated cylinder should now be upside down, full of water and with its opening under the surface of the water in the dishpan. Place the free end of the tubing from the plastic bottle inside the graduated cylinder. Your apparatus is now ready to trap carbon dioxide from the yeast (see Figure 3).

Figure 3. Picture of the inverted graduated cylinder gas collection apparatus.

d. You can test your gas collection apparatus by removing the tube from the bottle top and blowing gently into the tube. The bubbles you create should be captured inside the cylinder. (You will need to reconnect the tube to the bottle and re-fill the cylinder before starting your experiment.)

Testing Different Antifungal Medicines

In the second part of this procedure, you will be testing how effective different antifungal agents are on yeast. After following the steps in the "Setting up the Gas Collection Apparatus" section, you will be ready to test different antifungal agents. First you will grow yeast and collect the CO_2 produced without adding any antifungal agents. This will be your control. Then you will test two different concentrations of each antifungal agent.

1. Using the permanent marker, label each of the bottles with the name of the antifungal agent you will be testing, along with the concentration that you will test.

Label an extra bottle "control." When changing the concentration for one antifungal agent, you can re-use the bottle that you have used for the same antifungal agent before. Just make sure to start with the lowest concentration first, and rinse the bottle thoroughly between experiments.

2. Dissolve 1 teaspoon (tsp.) of sugar in ½ cup of warm water (110°F to 115°F, or whatever temperature the yeast package recommends). When the sugar is fully dissolved, add ½ tsp. of yeast, mix well, and pour into the appropriate bottle. *Tip:* It may help to use a fork to mix in the yeast. Be sure to note the actual temperature of the water in your lab notebook.
3. Cap the bottle tightly using the cap with the tubing attached. Make sure the open end of the collection tube is still inside the submerged gas-collecting graduated cylinder. Note the starting time in your lab notebook.
    a. There should be water in the tubing as soon as it is submerged in the water. The CO_2 gas will push some water out of the tubing before the graduated cylinder starts to fill with CO_2 gas.
4. Within five to ten minutes, the yeast should start foaming, and soon you should see bubbles collecting in the graduated cylinder. Note the time when you first start seeing bubbles in your lab notebook.
5. Decide how long to collect CO_2 (somewhere between 15–30 minutes is probably good, but you may need to adjust for your particular conditions). Use the same amount of time for all of your tests.
    a. *Note:* Do not let the graduated cylinder become completely filled with CO_2, but instead stop it before this point. If you let it become completely filled, and the next condition you test makes even more CO_2, this could lead to poor and inaccurate results because your graduated cylinder may fill up before your test time is over.
    b. *Tip:* If your solution makes a large amount of CO_2 very quickly, you can try to make it produce less CO_2 by using less sugar and possibly less yeast. For example, you could repeat this step using ½ tsp. sugar (instead of 1 tsp.) and ¼ tsp. yeast (instead of ½ tsp.).
6. When the time is up, note how much CO_2 was collected by observing how much water was displaced from the graduated cylinder.
7. Re-fill your gas collection cylinder, and carefully rinse out the yeast solution from the bottle. Repeat steps 2–6. You should run at least three separate trials for each testing condition.
8. Next, prepare your antifungal test solutions. You will be testing each antifungal agent at two different concentrations, a low concentration and a high concentration. The concentration range that you are testing is based on concentrations used in scientific studies listed in the Bibliography. You will be testing each antifungal agent and concentration one at a time (unless you decide to set up more than one gas collection apparatus).
9. First, check the "active ingredient" box on the antifungal medication's packaging and note what percentage of antifungal agent is in the medication. If you are using an antifungal medicine that has a concentration of antifungal agent greater than 1%, you will need first to dilute the medicine so that it is at a 1% concentration.
    a. For example, if the antifungal medicine is made up of 25% of antifungal agent, you will need to make a 25-fold dilution. You can do this by mixing 1/8 tsp. of the antifungal agent with 3 tsp. of warm water.
10. Start by preparing the *high test concentration* of the antifungal medicine. Take your medicine (or dilution) with 1% active ingredient (antifungal agent) and dilute it using the following steps to generate your high test concentration.
    a. First, dilute your antifungal medicine tenfold. To do this, measure 1/8 tsp. of antifungal medicine and mix this with 9/8 tsp. water (that is, 1 tsp. plus another 1/8 tsp.). Make sure you get all of the antifungal medicine off the measuring spoon. Using a toothpick may help. (Remember to wear latex gloves to protect your hands.)
    b. Mix this tenfold dilution very well. You may want to use a fork to vigorously stir, or whisk, the dilution. Mix it until almost all of the tiny pieces of antifungal medicine are not visible (although there may still be a small number visible).
    c. Next, measure ¼ tsp. of this tenfold dilution and add it to the ½ cup solution of warm water and sugar (as made in step 2, without the yeast). Mix well. What is the final-fold dilution of antifungal agent that you are using?
    d. Because you performed a tenfold dilution in step 10a, and a 100-fold dilution in step 10c, you will be using a 1000-fold dilution for these tests. See the Technical Note for calculations used to determine the concentration of the antifungal solution you just made.

Technical Note

By following steps 10a to 10c, you created a 1:1000 dilution of the antifungal agent, or an antifungal solution that is at a concentration of **10 µg/mL**. This will be your *high test concentration* in your test and can be calculated by doing the following measurement conversions and calculations.

In step 10a, you used 1/8 tsp. of antifungal medicine. The conversions below can be used to find out what 1/8 tsp. equals in grams (g).

Measurement conversions:

$$1/8\,tsp. = 0.62\,mL = 0.02\,fluid\,ounces\,(oz.) = 0.62\,grams(g)\,(with\,a\,density\,of\,approximately\,1\,g/cm^3\,for\,water)$$

We make the assumption that water and the antifungal medicine have similar weights. This is reasonable, because the medicine contains a lot of water.

This means that 1/8 tsp. of antifungal medicine weighs approximately 0.62 g. Consequently, you used 0.62 g of antifungal medicine in step 10a. This was in a total volume of 10/8 tsp., so in step 10c when you used ¼ tsp., you were using 1/5 of the 0.62 g, which is 0.124 g. 0.124 grams equals 124 milligrams (mg).

Because the medicine has the antifungal agent at a concentration of 1%, this means that the 124 mg of medicine you used only has 1% antifungal agent, or 1.24 mg antifungal agent. 1.24 mg equals 1240 micrograms (µg).

In step 10c, this 1240 µg antifungal agent was diluted into ½ cup water, and ½ cup equals 120 mL. If you divide 1240 µg by 120 mL, you get about 10 µg/mL, the final concentration of antifungal agent in the high test solution.

11. Next, make your *low test concentration* of the antifungal medicine.
    a. Use the 1000-fold dilution you prepared in the previous step.
    b. Measure out ¼ tsp. of the 1000-fold dilution and add it to another ½ cup solution of warm water and sugar (as made in step 2, without the yeast). Mix well. What is the final-fold dilution of antifungal agent that you are using? What is the final low test concentration of antifungal agent?
12. When you are done preparing both of your test solutions, start running the antifungal agent experiments.
    a. Start with the low test concentration experiment. Take the warm sugar solution with the low test concentration of antifungal agent and add ½ tsp. of yeast, mix well, and pour into the appropriate bottle. If the solution has cooled down too much, carefully heat it up to the right temperature again. Make sure not to heat it too much as this might affect the antifungal agent! Note the temperature in your notebook.
    b. Repeat steps 3 to 7, to test the antifungal agents.
    c. In step 4, you may not see foaming or the formation of bubbles. Why do you think this happens? Note in your lab notebook whether you see foaming or bubbling for each condition tested.
13. Once you have completed the low test concentration trials, rinse your bottle thoroughly and start the high test concentration experiments.
    a. Add ½ tsp. of yeast to the high test concentration solution, mix well, and pour into the appropriate bottle.
    b. Then repeat steps 3–7 again. If the solution has cooled down too much, carefully heat it up to the right temperature again before you start your experiment. Make sure not to heat it too much as this might affect the antifungal agent! Note the temperature in your notebook.
14. Run at least three separate trials for each antifungal agent concentration tested, and three separate trials of the yeast without any antifungal medicine added.
    a. Make sure to use the same water temperature each time you make a solution, because yeast activity is temperature dependent.
    b. Multiple trials help scientists make sure that their results are accurate and reproducible.

Analyzing Your Data

1. Calculate the average volume of the CO_2 produced for each amount of antifungal agent tested and write this in your lab notebook.

2. Make a graph of your results.
    a. Write the different antifungal medicines and concentrations tested on the x-axis (the horizontal axis).
    b. Plot the corresponding average volume of CO_2 produced on the y-axis (the vertical axis).
3. Which antifungal medicine was most effective at stopping the yeast from growing? Did any completely stop antifungal growth? Which was least effective? How do you justify your reasoning? Why do you think one worked better than another? Did any of the antifungal medicines have the same effect at both the higher and lower concentrations tested?