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FOOD BIOTECHNOLOGY - Fermentation Lab: Root Beer

Introduction

Classical biotechnology involves using microorganisms to ferment foods and beverages such as cheese, wine, beer, sauerkraut, kim chee, yogurt, buttermilk, root beer and others. Yeasts are fungi that produce carbon dioxide (CO₂) and H₂O in the presence of sugar. In this lab you will be using yeast to ferment and carbonate root beer.

For the first two days the yeast will use aerobic respiration (using O₂) to break down the sugar molecules and produce CO₂. This carbonates the root beer (fizz). Because the oxygen supply depletes, the yeast then turns to anaerobic fermentation (without O₂). The products of anaerobic fermentation give root beer its distinct flavor. The alcohol content of root beer is 0.03%. (It would take 100 root beers to equal 1 beer!)

Prelab Instructions Work in groups. Make several bottles (3-5) of root beer. In one, follow the protocol as written (this is the positive control) and in the others, decide what variable you want to experiment with and record your experimental protocol. For example, you can change the amount of sugar, yeast, the incubation temperature or time, or other factors related to the fermentation process. Change this variable incrementally in each of your bottles.

Prelab Questions

- 1) What is the expected outcome of each of your experimental samples? Include your rationale.
- 2) What controls should be done in the class and what is the purpose and expected outcome of each?

Materials for 1 bottle (380 mL)

Approx. 370 mL chlorine-free water (let tap water sit in open container overnight)
60 g sucrose
1.5 mL root beer extract (1500 uL)
liquid California Ale Yeast - about 2 mL (will be divided into smaller aliquots for the different bottles)
The yeast can be found at Wine/Beermaking shops or check out the web site at <http://www.whitelabs.com/>
Thermometer, hot plate
Sterile bottles with corks or rubber stoppers (Grolsch beer bottles with gaskets or plastic Nalgene bottles were recommended)

Procedure

1. Tape a label on each bottle with your name, date, period and identify which are the experimental bottles.
2. Determine where the 380 mL level is on your bottle using a suitable method. Mark this spot.
3. If water has not already been heated for you, heat water to 25-30°C using a hot plate or microwave oven.
4. If you have not already done so, wash your hands thoroughly with soap and warm water.
5. Pour sucrose into your sterile bottle.
6. Add some of the heated water and swirl until sucrose is completely dissolved, leaving enough room to add other ingredients so that the final volume is 380 mL.
7. Pipette the root beer extract into bottle and mix by swirling.
8. Using a sterile razor blade and blue micropipette tip, cut a tiny piece of the tip off, to slightly increase the diameter of the tip opening.
9. Pipette 500 µL of liquid yeast to each bottle.
10. Add enough of the warm water so that the final volume is 380 mL.
11. Cap tightly by stretching the rubber gasket over the ceramic stopper, and centering the gasket/stopper over the bottle as you clamp it down. Tip bottle to be sure it doesn't leak.

12. Incubate at 23-25°C (about 70-74°F) 7-14 days, then store in cooler dark place for at least 2-3 more days or up to 10 more days.

Results

Describe your root beer in terms of carbonation, odor, sweetness, yeastiness, and overall flavor. Try to quantify these characteristics using a rating scale. If it is less than acceptable, see if you can identify possible reasons for the problem(s).

Troubleshooting tips

- If yeast is not dissolved properly, little if any carbonation occurs.
- If water is too cold, yeast will lie dormant.
- If water is too hot, yeast could die.
- Too little yeast, little if any carbonation.
- Too much yeast, excess foaming, bottle could burst.

Questions (include in your conclusion in addition to the usual features)

- 1) 1. How does your experimental product compare to commercial root beer?
- 2) 2. Explain the role of aerobic respiration and anaerobic fermentation in this process. (Define each term, also)
- 3) 3. Which factor in the bottle do you think stops the production of root beer?

