RockEDU
SCIENCE OUTREACH

Testing Substrate Specificity in Yeast Fermentation

Fermentation of Food

Background

As Buchner discovered at the turn of the 20th century, the process of fermentation is a multistep, enzyme-catalyzed reaction. Inherent to maximal enzyme action is a defined set of optimal conditions and substrates. Therefore, the enzymes responsible for glycolysis and subsequent fermentation reactions will exhibit optimal reaction rates in an environment that mimics physiological conditions. To demonstrate this point, we will use "active dry" yeast packets from the supermarket (such as Fleischmann's or Red Star brands). This common product is a freeze dried collection of Saccharomyces cerevisiae, also known as "baker's yeast."

Naturally found on ripe fruits, like grapes, as well as on and in the human body, S. cerevisiae is a facultative anaerobe, and its biodiversity and carbon utilization is dictated by the carbon and energy sources available in its specific habitat. This demonstrates the incredible metabolic flexibility housed in these tiny eukaryotes, however, some substrates are more efficiently metabolized than others. In the absence of oxygen, S. cerevisiae will switch on its fermentation pathway as a mechanism to maintain a favorable cellular redox status (fermentation regenerates NAD+, which is essential for glycolysis), generating ethanol and carbon dioxide as byproducts.

To understand how different sugar substrates are utilized by S. Cerevisiae, we can measure the amount of CO2 produced. If you recall the stoichiometry for fermentation, for every mole of glucose, yeast cells will produce two moles of CO2, which makes a quantification of sugar metabolism fairly straightforward. While scientists have invented a number of devices to quantifiably measure the rate CO2 production resulting from fermentation in yeast, these devices are not practical for classroom settings. Here we will use a basic 15ml conical tube (such as Falcon or Corning brands) with gradation markings as a device to measure CO2 production in response to a variety of carbon sources.

Material List

- Baker's Yeast Packets (7% w/v in water)
- 40% w/v: Dextrose (glucose), Galactose, Lactose, Maltose, Sucrose
- 15mL Conical Tubes
- Needles
- Markers
- Pipette Tips
- Water Bath or Hot Plate
- Timers
- Beakers
- Micropipettes

Preperation

- Prepare sugar solutions at 40% w/v (40g per 100ml of H2O)
- Heat water baths and/or hot plates to 400C
- Warm all solutions to be used
- Puncture 2-4 holes in caps of 15ml conical tubes using a 21g needle

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- Immediately before experiment, prepare the 7% yeast solution in H2O

Procedure

1. Fill 15ml conical tube with 8ml of a sugar solution (recommended starting concentration is 0.5%, v/v)
2. Fill remainder of tube (~7ml) with 7% yeast solution such that the meniscus rises above the lip of the tube. NOTE: stock yeast solution should be agitated before adding to tube
3. Replace cap onto tube — because of holes, there will be a small squirt of solution to come out. NOTE: make sure that there are no sizeable air bubbles in the tube
4. Invert the tube, and place in a large beaker filled with water (preheated to 400C). Place this beaker into a water bath or onto a hot plate to maintain temperature NOTE: you can also test the effect of temperature on fermentation by adjusting temperature of water bath or hot plate
5. Immediately mark the bottom of the CO2 bubble (if there is one). Mark this point every at 5 minute intervals for 30 minutes.
6. At the end of the experiment, record the level of CO2 produced at each time interval

Discussion Questions

What conclusions can you draw about the metabolism efficiency of different substrates by S. cerevisiae?

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