Cellular Metabolism

Metabolism is the sum of all chemical reactions within the body. Our bodies can metabolize many types of molecules including glucose (simple carbohydrate) and glycogen (complex carbohydrate), lactic acid, lipids, and even proteins (amino acids). The metabolism of glucose is specifically termed "**cellular respiration**", which is the primary process by which eukaryotic cells use to create the high molecule **ATP (adenosine triphosphate)**. In cellular respiration glucose must first be broken down enzymatically in a process called "**glycolysis**" in order to release **pyruvate (pyruvic acid)** molecules contained in the molecular bonds. The pyruvate molecules can then follow one of two pathways (aerobic or anaerobic cell respiration) in order to generate ATP. Yeasts are some of the simplest eukaryotic organisms to perform this mechanism, and although not exactly like human cells, they are an important and useful model in cell biology. Most animal cells metabolize glucose in **aerobic cell respiration** (or respiration in the presence of oxygen), primarily through the Kreb's cycle and electron transport chain, in order to produce ATP molecules. Yeast can metabolize glucose and other sugars through a process of **anaerobic metabolism,** also known as "**fermentation**" (or cell respiration in the absence of oxygen), and then release CO_2 as a waste byproduct. This CO_2 waste is a valuable ingredient in food making processes such as bread and beer.

Artificial sweeteners are molecules that are derived from sugars structurally modified to elicit a sweet taste, but that do not present a significant calorie load. Two basic strategies are employed: either the sweetener is made so that it cannot be metabolized, or it is modified to have an extremely sweet taste so that only a very small amount is needed. The question for this lab is whether yeast metabolize artificial sweeteners in the same manner as they do natural sugars (or sucrose)? To approach this problem, we will compare the CO_2 production of yeast in response to sucrose and three other sweeteners. As shown in Figure 1 below, the sweetener **Splenda** (or **sucralose**) is chemically derived from sucrose, but it is 600 times sweeter and is actually structurally distinct from sucrose in that it contains 3 chloride (Cl) atoms and 5 hydroxyl (OH) groups whereas sucrose lacks Cl atoms and has 8 hydroxyl groups. **Equal** (or **aspartame**) is chemically structurally more similar to sucrose, with the exception of a methyl ester bond (OCH3), and is also 100-150 times sweeter. The natural sweetener **Truvia** (or **steviol**) was originally derived from the Stevia plant. Although very little is known about its metabolism in the human body, it is observed to be 300 times sweeter than sucrose and is structurally very different.

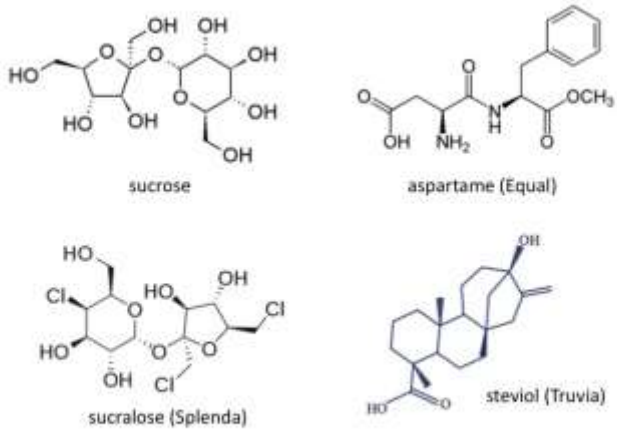

Figure 1. Chemical structures of nutrient molecules.

METHODS

Work in your lab groups. You will use respiration chambers and CO_2 probes connected to a computer program called *LoggerPro*. Make sure program data parameters are set to collect from 0 to 300 seconds, and the data sampling rate to 1 sample every 30 seconds. Make sure that the respiration chamber is clean and dry. Also, calibrate the CO_2 probe before every sample measurement to insure accurate data collection from a baseline. The instructor will prepare the yeast solution for you, and all other solutions your group will prepare, **one at a time**, by following instructions shown in Table 1 below. You will incubate each sample first for 5 minutes in a water bath and then transfer them to the respiration chamber to measure CO_2 production over an additional 5 minutes. To save time, begin incubating the next sample approximately 3 minutes after you have started recording CO_2 measurements of the previous sample. Allow a few minutes between experiments to air-out and re-equilibrate the probe.

[Instructor instructions for solution preparation]

2.5% Yeast solution = 2.5g yeast + 100ml H_20 [need ~75ml/lab]

5% Equal, Splenda, Truvia solutions = 5g + 100ml H_20 [need ~ 100ml of each]

5% Sucrose = 5g + 100ml H_20 [need ~200ml or 10g + 200ml H_20)

Table 1. Experimental conditions for the 6 experiments.

Experiment	Solution added to chamber	Yeast added to chamber
H_20	2 ml water	2 ml live yeast
Sucrose	2 ml sucrose	2 ml live yeast
Equal	2 ml Equal	2 ml live yeast
Splenda	2 ml Splenda	2 ml live yeast
Truvia	2 ml Truvia	2 ml live yeast
Boiled yeast + Sucrose	2 ml sucrose	2 ml boiled yeast

1. For the first experiment, pipet 2 ml of water into a clean tube.

2. Make sure to mix the yeast suspension by swirling, then pipet 2 ml into the same tube. Cap the tube and gently invert to mix solution. Place tube in water bath for 5 minutes.

3. After the 5 minutes of incubation, take tube out of water bath and pour contents carefully into respiration chamber. Wipe off any excess moisture on inside neck of chamber using a paper towel. **CAUTION: Do not directly immerse the probe in liquid; excess moisture will damage it!** Wash incubation tube thoroughly and leave on bench top to dry for next experiment.

4. Place respiration chamber on ring stand and secure. Calibrate CO_2 probe using straightened paper clip. Insert CO_2 probe gently but tightly into chamber. Hit "Collect" button at the top of the graph and record data in Table 2 provided below.

5. While experiment is running, one group member should prepare the next experiment for incubation. Follow directions for experiment #2 in Table 1 above and mix 2 ml yeast with 2 ml sucrose in incubation tube and place in water bath. This will be ready for CO_2 measurement by the time experiment #1 is done. **Clean & dry chamber thoroughly and allow chamber and probe to re-equilibrate (return to near starting levels) before beginning next experiment.**

6. Repeat steps 1-5 for the rest of the experiments. For each experimental group calculate the Δ CO_2.
Δ CO_2 = CO_2 at 300 sec – CO_2 at 0 sec. (The difference between the measurements allows standardization of data among different probes and software at each of the tables.)

Table 2. Measurements of CO_2 gas production (ppm) over time (sec) for experiments 1-6.

Time (sec)	CO_2 gas level (ppm)					
	H_20	Sucrose	Equal	Splenda	Truvia	Boiled yeast/sucrose
0						
30						
60						
90						
120						
150						
180						
210						
240						
270						
300						
Δ CO2						

When the class has completed running their Cell Metabolism experiments we will compile the class data in Table 3 (below) and perform the following analyses:

Analysis of Class Data of ΔCO_2 among experimental groups:

1. We will use Microsoft Excel to calculate average CO2 production for each treatment group. Record the average CO2 production in Table 1.

2. We will then use a One-Way ANOVA to calculate whether average CO2 production differs significantly among the treatment groups. Record the P-Value from the ANOVA belowTable 1.

3. Numerically rank, from highest to lowest (1 = highest, 6 = lowest), the treatment groups according to their average CO2 production.

Table 3. Summary of Class Data for Cell Metabolism Lab						
	CO_2 Gas Produced by Fermentation (ppm)					
Variable	**H_20**	**5% Sucrose**	**5% Equal**	**5% Splenda**	**5% Truvia**	**Heat-Killed yeast + 5% Sucrose**
Table 1						
Table 2						
Table 3						
Table 4						
Table 5						
Table 6						
Avg ΔCO_2 ppm						

Questions:

1. Which molecule(s) appear to be metabolized best by yeast? Which molecule was metabolized the least?

_____

2. Which artificial sweetener was most similar to sucrose in CO_2 production? _____

Why do you think it was similar (based on our discussion)? _____

3. A) What parameter did you measure in this experiment (the "dependent variable")? _____

B) What parameter did you change between experiments (the "independent variable")? _____

4. What was (were) the: A) Positive control(s) in the experiment? _____

B) Negative control in the experiment? _____

5. Under some conditions, certain types of yeast, like *Candida albicans*, can be pathogenic. Individuals prone to yeast infections are recommended to avoid sugars in their diets. Based on our experimental findings which sweetener would you recommend a patient use to reduce susceptibility to yeast infections?