Kombucha is a **symbiotic** culture of various species of yeasts and bacteria. As a result of various metabolic pathways, including fermentation, an extremely acidic solution (pH 2-3) is produced, also having around 1% alcohol. The major acid is acetic acid, and a host of other chemicals including a few vitamins. Many unfounded claims revolve around the tea---cures for cancer, for hypertension, AIDS, and so on. The purpose of making this stuff in lab is for you to see symbiotic microbial relationships in action, and identify some of the variables that can affect the relationships.

The best carbon source for the Kombucha is glucose, which is a **monosaccharide**. Disaccharides are cheaper, but special enzymes are necessary to split them into monosaccharides. Each table will pick a particular sugar to use in this exercise. **Glucose** will be run along with several **disaccharides**:

- Sucrose (enzyme: sucrase)
- Maltose (enzyme: maltase)
- Lactose (enzyme: beta-galactosidase)

**OBJECTIVES:**

Grow a culture of kombucha.
Learn about symbiotic relationships between microorganisms.
Determine differences in growth based on nutrient source.

**MATERIALS NEEDED:**

**per table**
- 500 ml boiled water
- Kombucha colony
- bags of black tea
- thermometer
- weight balance
- 1 kind of sugar
- plastic container and lid

**AFTER INCUBATION:** weight balance and pH meter

**THE PROCEDURE:**

1. Make tea using 1 black tea bag (~5 grams), 40 grams of white sugar, and 500ml of distilled water.
2. Heat the water, to boiling, to infuse the tea leaves.
3. **Cool** to room temperature (25 degrees C) if boiled water is used. IF using distilled water, you might want to heat it a bit so that the sugar and tea will go into solution better
4. Weigh out 20 grams of the Kombucha colony.
5. Add the Kombucha colony and 50ml (3-4 Tbs.) of previously prepared Kombucha tea to a plastic container with a lid. This will give a final sugar concentration of about 7.5%.
6. Cover the container with the lid (loosely) to keep out insects and air-borne contaminants.
7. Place it on the porcelain tray in the front of the room. The cultures will incubate at room temperature in the back.
8. Let it ferment for about 8-10 days.
9. **AFTER INCUBATION:**
   - Weight out the whole Kombucha colony and compare it against the original 20 g weight.
   - Determine the pH of the tea.
   - Compare the tea pH and weight of the colonies among the various sugar sources.
   - Make a smear of the colony and do a simple stain. Look at it with 100X.

**INTERPRETATION:**

Acetic acid is a major end product of this symbiotic culture.

**QUESTIONS:**

1. Define symbiosis.

2. Most food or drink would be rapidly destroyed by contaminant bacteria, but kombucha is not. What is there about kombucha that reduces bacterial contamination?

3. Which sugar is the best for the growth of the symbiotic culture?

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8/2009, Jackie Reynolds, Richland College