MICROBIOLOGY 101 LABORATORY MANUAL

EXERCISE #16: PREPARATION OF YOGURT, BEER AND SAUERKRAUT

NAME, ID #:_____

TA Name _____

REVISED: 08/04/99

INTRODUCTION:

MICROBIAL MANUFACTURING OF FOOD

As you will learn in the final section of the lecture, many of our favorite foods are the result of "**POSITIVE or CONTROLLED *SPOILAGE***". That is, fresh food supports the growth of one or more microbes that modify the character of the food in some fashion that humans **FIND APPETIZING**. Since such microbial-produced foods are ancient, they were all undoubtedly discovered **BY ACCIDENT**. That is, a food that "spoiled" was never-the-less eaten by some starving human (or possibly a hungry teenager) who found that they actually liked the flavor of the modified produce (remember your first taste of beer, cottage cheese or limburger cheese?). In most cases the food is modified as a result of **ANAEROBIC FERMENTATION** which produces a mixture of organic wastes including organic acids, (e.g. formic, propionic, acetic & lactic), that lower the pH, various amines, that raise the pH, esters and/or ethanol. These modified foods were observed to be "preserved" in that they remained in an eatable condition for long periods at room (cave) temperature. That is, the low pH and ethanol prevented most other microbes from growing on and destroying the value of the fermented food. Examples of such foods include pickles, ethanol based drinks (beer, wine, mead etc.), cheeses, butter milk, yogurt, soy sauce and sauerkraut. In this exercise you will prepare beer, yogurt and sauerkraut (German = acid cabbage) by fermentation.

YOGURT

Yogurt is produced by the fermentation of warm milk by ***Lactobacillus bulgaricus*** and ***Streptococcus thermophilus***. These two bacteria are able to grow at 40-45^{o}C, during which they produce lactic acid and various other byproducts that give yogurt its unique flavor.

- Straight yogurt generally tastes bitter due to the aldehydes present in it, so fruits, honey or other flavoring agents may be added to "take the bitter edge off".
- Yogurt is a concentrated protein product that has been enriched by additional vitamins produced by the microbes. Some people feel that the ***Lactobacillus spp***. help settle digestive problems and there is data to support that yogurt can help control yeast infections of the gastrointestinal tract and the vagina.
- Yogurt is used as a douche to replace normal vaginal lactobacilli flora killed by antibiotic treatment of vaginal infections.
- Yogurt also contains antibiotics produced by the microbes.

BEER AND WINES

Beer and wines are probably one of the most ancient fermented foods used by man. Beer and wines are produced when the sugars in plants are **ANAEROBICALLY FERMENTED**, usually by yeast. In the absence of oxygen the sugars are metabolized to yeast pee (better known as ethanol, booze, joy juice, rot-gut) and carbon dioxide (produced by yeast flatulence). Some acid is also produced resulting in a low final pH. Wine and beer can be made from any plant material that contains free sugar, but we generally equate wine with grapes and beer/whiskey with grains.

A FAQ about wine/beer is "***why are there so many different varieties and flavors of beer & wine (even made from the same grains or vineyard)?***"

- **ANSWER**: The answer lies in the **SUBTLE COMPLEXITY** of nature which operates at many levels in the case of beer & wine fermentation. For one thing the **VARIETY (STRAIN) OF YEAST** used to ferment grain, grape or other carbohydrate sources (berries or even dandelions) varies greatly and each strain (yeast mutant = variety = strain) alters the flavor of the wine according to the slightly different mix of metabolic by-products produced by each genetically different yeast strain. Second, as you are aware, the grain & grapes themselves **VARY GENETICALLY**, so the unique starting chemicals within each strain of grapes is reflected in the distinct flavor of the wine that results from their fermentation. Then there are the subtle and numerous variations in **SOIL COMPOSITION, WATER CHARACTERISTICS** and the **WEATHER** from year to year. Finally, the **CONDITIONS OF FERMENTATION** (time, container composition, temperature) also affect the final flavor. A difference in a few degrees of temperature, the wood used in the fermentation vessel or a few more days of rain (or sun) can significantly affect the grain or grape's chemical composition (e.g. sugar and associated chemicals) and the subsequent flavor of a beer or wine produced; hence the enormous attention paid to the "YEAR" a wine was produced. Even the **LOCATION** of the vines on a hill side will effect a wine's flavor.

SAUERKRAUT

Sauerkraut is produced from the fermentation of cabbage by a group of bacteria that produce mostly **LACTIC ACID** as an end product of the metabolism of the nutrients in the juices of the cabbage. The bacteria involved are ***Leuconostoc sp***. and ***Lactobacillus sp***. The "sp." refers to the fact that many strains of these organisms exist and each one produces sauerkraut with a slightly different flavor; much like the different beer, wine and cheese flavors. These bacteria grow sequentially. Initially the ***Leuconostoc sp***. produces organic acids, which lower the pH to about 3.5 and the chemical **DIACETYL** (the flavor of butter). They are followed by ***Lactobacillus sp***. which produce more acids, lowering the pH to ~2.0. To make sauerkraut the cabbage must be shredded to produce a large surface area for the growth of the microbes and to extract the plant juice-nutrients which will be metabolized by the microbes. Sodium chloride (table salt) is added to a concentration of 3% to provide **OPTIMUM** environmental conditions for the growth of the desired fermenting bacteria, to help **EXTRACT** the tissue juices, and to **INHIBIT** the growth of microbes (molds) that would ruin the cabbage. The cabbage/salt mixture is weighted down to squeeze out the juices and incubated at room temperature in covered containers. The covers inhibit the **entry of oxygen** into the mixture and allows the ***anaerobic fermentation*** to occur. At the end of the fermentation period the pH should be ~ 2.0 and the sauerkraut should contain about 1% lactic acid.

SAUERKRAUT PREPARATION PROCEDURE:

1. Read pg. 128-30 in ***A Photographic Atlas for the Microbiology Laboratory*** for a description of the biochemistry of the fermentation of the cabbage. You should know what the important chemical byproducts of the fermentation process are that are responsible for the flavor and character of the sauerkraut, but you do not need to learn the biochemical pathways on these pages.
2. The instructor will demonstrate how to core, weigh slice and prepare the cabbage for fermentation. Remember to use sanitary techniques throughout the procedure.
3. Write down a DETAILED DESCRIPTION of the actual process as if you were going to go home and make your own sauerkraut (surprise you parents with what you've learned in college).
4. After fermenting for 5 weeks you will have a hot dogs & sauerkraut party.

PURPOSE OF THIS LABORATORY PORTION:

1. To make yogurt and beer.
2. To understand the effects of fermentation on food.

RELATIONSHIP TO LECTURE MATERIAL

- NetText101/**102**: CHAP. XX.

BEER PREPARATION PROCEDURE

1. Dissolve the contents of one package of prehopped malt powder in 1.5 liters of water.
2. Bring the mixture to a boil. It will foam because of the protein content.
3. Cover the pot and cool the mixture rapidly to 120^{o}F
4. Bring the volume to 1 gallon with sterile cool water.
5. When the temperature is 70-72^{o}F add the yeast directly to the wort.
6. Check the wort with a hydrometer and write the number on a label for the fermentation vessel. The initial reading should be about 1350.
7. Fill the labeled sterile fermentation vessel with the wort.
8. Add a trap to the vessel. The trap consists of a rubber stopper with an attached length of latex tubing. The end of the tubing is inserted in a flask of water so the gas formed during the fermentation bubbles off in the water.
9. Ferment the wort down to a reading of about 1011. This should take 5-7 days. Look for the same low hydrometer reading 2 days in a row.
10. It is now time to bottle the beer. Add one level ½ teaspoon of corn sugar to each sterile 12 oz. bottle. Fill with beer and cap.
11. Give each bottle a shake to make sure the sugar is dissolved.
12. Condition the beer at the same temperature as the fermentation for 7-10 days.
13. At the end of the conditioning period either chill and drink or store in the refrigerator until tasting time.

ALTERNATIVE: WINE PREPARATION PROCEDURE

1. Take 2/3 gallon jar of apple cider and add 100-150 gm of glucose.
2. Mix until dissolved.
3. Add 10 ml of yeast culture and mix.
4. Stopper with tubing and let it sit for 5 weeks in a cool dark place.
5. Taste appropriately at the end of the semester. The wine can only be "tasted" because of legal restrictions.

YOGURT PREPARATION

The general process, which many people do at home is as follows:

- Milk (from any mammal; usually Pasteurized & low fat) is scalded (brought just to a boil) to kill contaminating microbes and to thicken it. Powdered milk may be added to thicken it.
- After cooling to 40-45^{o}C the milk is inoculated with a STARTER CULTURE containing the required microbes.
  - Commercial brands use their own cultures.
  - Home-made yogurt starter cultures may be purchased from health food stores or obtained from commercial brands (unpasteurized).
  - In under-developed countries the tradition has been for 1,000s of years to keep a pot of yogurt going continuously near the kitchen fire. As the yogurt is eaten the housewife adds a fresh milk-mixture: ***imagine eating yogurt that has been continuously cultured for perhaps 10,000 years***.
- The mixture is incubated at 40-45^{o}C for 12 to 18 hours at which time it may be eaten or stored in the cold.
  - Heating can be done on a stove or using a commercial incubator made for the purpose.
  - The lactic acid lowers the pH and cuddles the milk protein, giving it a gel-like consistency.

 **SAMPLE QUESTIONS: You should be able to answer these questions at the conclusion of this laboratory.**

- Explain the purpose of the salt in the fermentation of sauerkraut?
- Why is the cabbage covered up with the water-filled plastic bags?
- Why does the wine/beer stopper have tubing in it? What would happen if you used a plain, solid stopper to tightly seal the jar?
- List three factors that can affect the character of a wine or beer.
- Suggest a scenario leading our ancestors to discover cheese; Yogurt.
- What does lactic acid do in the production of sauerkraut?
- What is the purpose of the corn sugar added to each sterile bottle before adding the beer and capping?

- Recently two workers in a brewery climbed down into a recently emptied fermentation vat to fix something. A brief period later they were both found dead. What killed them?

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