Exercise 12-A
FOOD MICROBIOLOGY - FERMENTATION
(SAUERKRAUT, WINE & ROOTBEER)

Fermented Foods - Sauerkraut

Fermentation may be defined as the anaerobic decomposition of organic compounds that involves an organic compound (made within the cell) as the final electron acceptor. When the final electron acceptor is **pyruvic acid** ($CH_3COCOOH$), it picks up 2 electrons and 2 hydrogen protons to form **lactic acid** $CH_3CH(OH)COOH$, thus lactic acid is a common end product of fermentation. An enzyme called **lactate dehydrogenase** catalyzes reactions converting pyruvate (CH_3COCOO^-) to lactate ($CH_3CH(OH)COO^-$) as it converts NADH + H^+ to NAD (both reactions are reversible). Bacteria that use this enzyme form lactic acid as their only fermentation product, so are **homofermentative**, and are often called **lactic acid bacteria**. Fermentations involving such organisms have provided a means of preserving and flavoring foods that has been used by humans for centuries (long before microorganisms were recognized as participants in the process).

Sauerkraut is a form of fermented food made from cabbage using lactic acid bacteria. Sauerkraut is prepared by packing alternate layers of salt and shredded cabbage into a container, placing a weight on it, and allowing it to ferment. The salt creates a hypertonic environment that causes juice to be withdrawn from the cabbage. This forms the **brine** that helps to maintain the anaerobic conditions required for fermentation. Lactic acid bacteria associated with the cabbage grow and produce acidic end products that inhibit the growth of other bacteria that would cause spoilage. Ultimately, the cabbage is preserved and flavored by the fermentation process.

Procedure (Work in groups of 5-6 people or table groups)

1. Obtain a section of fresh cabbage, weigh it and then shred it **finely**. If the cabbage is not cut into small enough pieces, there will be inadequate exposure of cut surfaces to the salt added, and poor brine formation. If air remains between the cabbage shreds, fungal growth will ruin the product.

2. Weigh out a quantity of salt equal to 3% of the cabbage weight (multiply your cabbage weight by .03), sprinkle this over the shredded cabbage, mix it, and then place it in a 600ml beaker.

3. Compress the mixture by placing a weight (400ml beaker filled ¾ full of water) on the surface, and allow it to sit until a layer of juice has covered the cabbage. **Note** – Do not press on the weighted beaker with your hands as this might result in injury.

4. Align the pouring extensions (beaks) of the inner and outer beakers, cover the container with plastic wrap and secure this with a rubber band. Allow the sauerkraut to incubate at room temperature until brine is formed. Test the pH of this juice with pH paper and record the pH.

5. Incubate the sauerkraut at room temperature for two to three weeks, and then examine the finished product. If fungal growth is not evident, smell and taste the sauerkraut, record descriptions of aroma and flavor, and again check the pH.

6. OPTIONAL – Obtain a plate of Bromocresol purple (BCP), lactose agar and streak the surface with a sample of material taken from the sauerkraut container (avoid fungi). Incubate the plate at room temperature until the next laboratory session.

7. Remove a segment of cabbage from the finished sauerkraut, and make a smear on a clean glass slide by gently scraping the surface with a sterile loop (alternatively – obtain a sample of cell material from your BCP lactose agar plate). Make a Gram stain of this material, being careful not to over decolorize (a smear made from a leaf scraping will be very thin). Examine the smear and sketch the cells present. You should see both Gram-positive cocci and Gram-positive bacilli (rods).

   Note – The types of fermentative organisms present within the sauerkraut mixture will change over time. Sauerkraut should include *Leuconostoc mesenteroides*, *Lactobacillus brevis*, and *Lactobacillus plantarum* to attain the proper aroma and flavor; however, other lactic acid bacteria such as *Lactococcus lactis* may be present.

Fermented Beverages - Apple wine and Root Beer

The type of fermentation a food or beverage undergoes is determined primarily by the type of fermentative organisms present, but is also influenced by the initial pH and carbohydrate content of the material being fermented. Foods rich in carbohydrates, low in acid, and well buffered, commonly undergo lactic acid fermentation involving bacteria; while foods rich in carbohydrates, highly acidic, and poorly buffered are conducive to alcoholic fermentation by yeasts.

Most fruit juices are high in fermentable carbohydrates, poorly buffered and relatively acidic. At temperatures of 20 - 30^{o} C these juices readily undergo alcoholic fermentation to yield a product called wine (also called cider or perry when apples or pears are used). Fermentation involving the common brewing yeast, *Saccharomyces cerevisiae*, is sometimes diagrammed as follows:

$C_6H_{12}O_6$ ----------> $2\ C_2H_5OH + 2\ CO_2$
hexose sugar — ethyl alcohol — carbon dioxide

This equation shows the end products formed, but does not address the final electron acceptor involved. In an alcoholic fermentation, pyruvate (CH_3COCOO^-) is decarboxylated to yield acetaldehyde (CH_3COH) and carbon dioxide (CO_2). This reaction involves the enzyme **pyruvate decarboxylase**. The **acetaldehyde** then serves as the final electron acceptor, allowing NADH + H^+ to be oxidized to NAD (so it can be used again). The acetaldehyde picks up 2 electrons and 2 hydrogen protons to form ethanol (ethyl alcohol = CH_3CH_2OH or C_2H_5OH). Organisms able to form multiple different end products through fermentation are **heterofermentative**.

If the carbon dioxide (CO_2) is considered a waste gas and is allowed to exit the fermentation chamber, the finished product (wine, cider, etc.) will contain ethanol, but will lack bubbles. A sparkling wine (Champagne) can be made by allowing wine to undergo a secondary fermentation within a sealed bottle to create carbonation. The production of root beer and other **carbonated** or "sparkling" beverages also requires that fermentation occur within a sealed container, so that the carbon dioxide is not allowed to escape. Such containers must be cooled prior to opening, or the **effervescence** (fizzing or foaming due to escaping gas) will cause much of the fermentation product to be lost.

Apple Wine or Cider Procedure:

1. Aseptically pour 100-200 ml of the juice provided into a sterile Erlenmeyer flask. Be careful to leave enough space in the flask to prevent the foam produced during the early stages of fermentation from clogging the gas trap.

2. OPTIONAL: Add 5-10 grams of sucrose (table sugar) to the juice sample and encourage it to dissolve by gently swirling the flask.

3. Determine the specific gravity of the juice sample using a hydrometer as demonstrated, or measure the degrees Brix (°Bx) using a refractometer as demonstrated. The table below can then be used to estimate the percentage of sugar and/or the percentage of alcohol likely to be formed.

4. Inoculate your juice sample with *Saccharomyces cerevisiae* by adding a small amount (1/8 tsp.) of active dry yeast to a tube of sterile distilled water, mixing by gently swirling, and then pouring this into the juice after it has been allowed to stand for about five minutes.

5. Stopper the flask and connect a gas trap (rubber tubing plus a test tube approximately 3/4 full of water or a plastic device specifically designed for this purpose).

6. Incubate the preparation at room temperature for about two weeks. Observe the flask frequently during this incubation period and notice the change in yeast population over time. Notice also the amount of carbon dioxide gas being produced.

7. When the yeast appears to have stopped fermenting, i.e., when strings of gas bubbles are no longer visible within the flask, carefully pour or siphon the fermented juice into a new clean sterile flask. Avoid carrying over sediment if possible. This step is called **decanting**.

8. Check the aroma of your preparation. (You may also check the flavor, but do so only if you were very careful about aseptic technique during the preparation stages.) At this point it will be necessary to combine the juice samples from several groups so that each new flask is filled to within a few millimeters of the top. This will decrease the extent to which the "wine" is exposed to air. Reconnect the gas traps and allow the juice to continue incubating at room temperature.

9. Approximately 1 month (or as indicated by your instructor) after inoculation, examine your preparation again and determine the specific gravity as before. Use the table below to estimate the percent alcohol in the final sample.

   Note - as an alternate method you may use an alcohol hydrometer to determine the ethanol content of you fermented juice product (wine).

Table 12-A.1
The Relationship of Specific Gravity to the
Estimated Percentages of Sugar Content and Alcohol Formation

Specific Gravity	Percent Sugar (°Bx)	Alcohol Value (%)	Specific Gravity	Percent Sugar (°Bx)	Alcohol Value (%)
1.000	0.0	0.0	1.090	22.5	12.0
1.010	3.0	1.0	1.100	24.5	13.0
1.020	5.0	2.0	1.110	26.5	14.5
1.030	8.0	4.5	1.120	28.5	15.5
1.040	10.5	5.0	1.130	31.0	17.0
1.050	12.5	6.0	1.140	33.0	18.5
1.060	15.0	7.5	1.150	35.0	20.0
1.070	17.5	9.0	1.160	37.0	21.5
1.080	20.0	10.5			

Root Beer Procedure:

1. Working as a single laboratory group, weigh out 454 grams of sugar and pour this into a clean one-gallon container. (If a container with a pour spout is available, this will make filling the bottles easier.)

2. Fill the container about ¾ full with tepid tap water and stir or secure the lid and shake the container briskly to dissolve the sugar.

3. Thoroughly mix the contents of one bottle of root beer extract and using a sterile 10ml pipette, transfer 14.75ml of extract into the sugar solution. This transfer will require the pipette be partially filled more than once, and that the dark-colored extract be rinsed out between transfers. Mix the extract and sugar solution by stirring or shaking the container. At this point the "root beer" will taste much like warm root beer-flavored Kool-aid.

4. Add 1/4 teaspoonful of active dry yeast to a tube of sterile water and allow it to stand for 5 minutes. Mix this yeast-water combination by rolling the tube between the palms of your hands, and then add it to the "root beer".

5. Mix this combination of ingredients well to distribute the yeast, and then carefully fill the remaining space within the container with clean water.

6. Carefully pour the root beer solution into clean glass bottles (thick-walled bottles such as champagne bottles are recommended). Fill each bottle to within 1/2 inch of the top. Use the bottle-capping device provided to cap the bottles as demonstrated.

7. Incubate the mixture at room temperature for about two weeks (or until it becomes effervescent). When the root beer has become effervescent, move it to a cool location (or refrigerator) to prevent the bottles from leaking or breaking (exploding) due to excess carbon dioxide build-up.

8. **REFRIGERATE PRIOR TO OPENING!** - Examine the fermentation product. Test the flavor and aroma and record your findings.

<u>Questions</u>:

1. The type of fermentation a food or beverage undergoes is determined primarily by?

2. The fermentative bacteria responsible for the formation of sauerkraut are? What fermentation product is responsible for the flavor of the sauerkraut?

3. The fermentative organisms used in the production of our "wine" and root beer were? Are these organisms homofermentative or heterofermentative?

4. The metabolic pathway used by *Saccharomyces* in the fermentative production of wine and root beer yields what end products?

Exercise 12-B
FOOD MICROBIOLOGY - CULTURED FOODS
(CHEESE & YOGURT)

Microbiology of Cultured Foods - Cheese

Cheese making basically involves the adding of a starter culture of bacteria to milk and letting the mixture incubate for a period of time. Then a proteolytic enzyme (**rennet**) is added to coagulate the solids (mostly milk protein **casein**). Finally the coagulated milk solids (**curd**) is separated from the liquid (**whey**), pressed to squeeze out much of the water, and wrapped in cheesecloth to dry. The nature of the bacteria used as a starter is an important factor contributing to the variety of cheeses. Other factors include the temperature of manufacture and the presence or absence of a secondary microbial population within or on the cheese surface. Cheeses may be classified as **soft** (having from 50-80% water, **semi-soft**, or **hard** (having 40% or less water) ripened or unripened. An **unripened** cheese is a finished product as it comes from the initial processing steps (e.g., cottage cheese). A **ripened** cheese is one which is aged or on which a secondary microbiota is encouraged (e.g., cheddar or blue cheese).

Note – The table of well-known cheeses included in this exercise is adapted from *Scientific American*, September 1981. Due to changes in taxonomy (1986), organisms formerly identified as *Streptococcus lactis* and *cremoris* are now classified as *Lactococcus lactis*, subspecies *lactis* and *cremoris*.

Making an American Type Cheese (Adapted form U. C. Cooperative Extension sheet #273)

Procedure:

1. Add 2 cups of commercial cultured buttermilk to 5 gallons of sweet pasteurized milk (You may use either whole or low-fat milk). If a smaller volume of milk is used you may reduce the volume of buttermilk accordingly, but the amount of starter culture used is not critical.

2. Mix thoroughly and heat the mixture to 85^{o}F (37^{o}C). Avoid applying excess heat as this will inhibit the activity of the enzyme added.

3. Dissolve 1 cheese rennet tablet or two junket tablets in 1/4-cup cold water and stir thoroughly into the milk. Alternatively, add 6-8 drops of liquid rennet solution to the milk and stir it in.

4. Let the mixture stand until a firm curd forms, about 30-60 minutes. Then cut the curd into cubes of approximately 1/2-in. and allow it to stand 3 min. without stirring.

5. Heat the curd slowly to 100-105^{o}F. Stir slowly throughout the heating period (about 30 min.) to prevent the cubes of curd from sticking together and forming lumps.

6. Keep the curd at 100-105^{o}F for about an hour and a half or until it becomes firm. (An indication of firmness is a squeaky sound produced when the curd is chewed.)

7. Pour the whey and curd through cheesecloth over a large pan and retain the curd.

8. When the curd has cooled to about 90^{o}F, sprinkle four tablespoons of salt over it and mix thoroughly (If a smaller volume of cheese is being made, reduce the salt accordingly.) Let this mixture stand at room temperature until it forms at mat (about 1 Hour). Cut the matted curd into 1-inch cubes.

9. Place the curd on cheesecloth (3-4 thicknesses of cheesecloth) wrap tightly and form the curd into a ball. Then press it down to form a circular disk.

10. Lay a piece of wet cheesecloth over the curd and place a weight on it. Press the curd for about 1/2 hour.

11. Remove the weight, and recover the cheese with new cheesecloth that has been wetted to insure a smooth fit. Then press the cheese again for 8-10 hrs.

12. Place the cheese in a cool dry place for 4-5 days. Rub the surface of the cheese with salt 2 days in succession and turn occasionally until it forms a rind.

13. When the surface is dry, (4-5 days) cover the cheese with paraffin. Cure the cheese in a clean dry place (50 - 55^{o}F.) for 4 to 6 weeks.

The Microbial Populations of Cheese and Other Foods

Many microorganisms are responsible for the aromas, flavors, and other characteristics of various cultured foods. Although the final identification of these microorganisms is not possible here, the general nature and characteristics of several types of organisms can be observed.

Procedure:

1. Obtain a plate of Bromocresol purple (BCP) lactose agar and using a sterile loop, slice the agar into two equal halves. You may find it useful to mark the back of the plate with a glass marker first to indicate where the agar is to be cut.

2. Use a sterile loop to transfer a small portion of buttermilk onto one side of the plate, and a small portion of yogurt onto the other. Be sure to use a streaking method that will yield well-isolated colonies.

3. Place your plate into the anaerobic chamber or zip-lock bag provided, and incubate them at 37^{o}C for 24 hours, and then at room temperature until the next laboratory period.

4. Observe the colonies that develop. Compare their characteristic shape, size, and color as well as any color changes that may be evident in the medium. **Remember** that Bromocresol purple is a pH indicator.

5. Gram stain one or more of the colonies from each side of the plate to further characterize the organisms present. Gram-positive cocci in chains (streptococci) will occur on the buttermilk side, while Gram-positive cocci and bacilli are common on the yogurt side.

6. Observe the texture, aroma, and flavor of the various cultured food products provided. Note the distinctive characteristics of cheeses that are soft, semi-soft, hard, ripened and/or unripened. Compare the various cheese names to the charts provided and try to identify which of the organisms present are responsible for the most distinctive flavors and/or aromas. Compare the commercial yogurt to that which was "home made". Record your findings on the data sheets provided.

Microbiology of Cultured Foods - Yogurt

Yogurt is produced with a yogurt starter that is usually a mixed culture of ***Streptococcus thermophilus*** and ***Lactobacillus bulgaricus*** in a ratio of 1:1. (In the new edition of the Bergey's Manual Vol. 2, the species name *bulgaricus* has been reduced to the rank of subspecies due to DNA homologies, and these rods are now identified as *Lactobacillus delbrueckii* subspecies *bulgaricus*.) During the early stages of yogurt formation, the cocci present grow faster than the rods, and are primarily responsible for acid production (**lactic acid**), while the rods add flavor and aroma. The associative growth of the two organisms results in lactic acid production at a rate greater than that produced by either of the two when grown alone, and more **acetaldehyde** (the chief volatile flavor component of yogurt) is produced by *L. bulgaricus* when growing in association with *S. thermophilus*.

The first step in yogurt preparation is reducing the water content of either whole or skim milk by at least one-fourth. This is easily accomplished by adding approximately 5% by weight milk solids or condensed milk to a sample of ordinary milk. The concentrated milk is then heated to 82-93^{o} C for 30-60 minutes, and then cooled to around 45^{o}C. The yogurt starter is then added, and the yogurt is incubated at 45^{o} C for 3-5 hours. The finished product is usually cooled prior to consumption, and typically contains around 10^{9}organisms per gram.

Making Yogurt at Home (Adapted, from U. C. Cooperative Extension leaflet #2722.)

Equipment:

Double boiler that holds 5 cups.
Food thermometer with a range of at least 100 to 200^{o}F.
Sterilized container that holds at least 5 cups (glass jar, crockery or stainless steel bowl) or five sterile one-cup containers.
Cake pan, electric skillet, yogurt maker, or other device that can maintain the culture at the required temperature for incubation.

Yogurt Recipe:

1 quart pasteurized milk (whole, low-fat, or nonfat)
1/3-cup nonfat dry milk **or** 13-ounce can evaporated milk
1/3 to 1/2-cup commercial, unflavored (or vanilla) yogurt
Optional: 1-1/2 packages unflavored gelatin

Procedure:

1. Add nonfat dry milk or evaporated milk to the quart of fresh milk. Optional: If you prefer a firmer yogurt, dissolve the unflavored gelatin in the cold milk. You could also heat the milk mixture to 180^{o} F in the double boiler and hold the milk at that temperature for up to 45 minutes. The longer the time, the firmer the finished yogurt will be. Keep the double boiler covered during the heat treatment. Cool the milk to 120^{o} F.

2. If you did not use the heat treatment described above, heat the milk to 120^{o} F. If you added gelatin be sure it is completely dissolved. Cool the milk to 110^{o} F.

3. Remove 1/2 cup of the warm milk (110^{o} F) and thoroughly blend it into the commercial, unflavored (or vanilla) yogurt. Blend this mixture into the remaining warm milk.

4. Pour the milk and yogurt mixture into the large sterile container and cover it, or put it into clean 1/4-pint bottles or other small containers and cover these with loose-fitting caps. (Clean custard cups or jelly glasses are satisfactory for home use, because the yogurt can be eaten directly from the container in which it is made.)

5. Place the container(s) in a pan of water maintained at 110^{o} F. Let the milk ripen at that temperature until it has thickened and has a tart, acid flavor. The ripening period usually takes at least 3 to 6 hours. If you prefer a sharper flavor, lengthen the incubation time. Yogurt made in a commercial yogurt maker may be incubated over night.

6. Remove the ripened yogurt from the "incubator" and chill it in the refrigerator. Refrigerate the yogurt until it is used. Yogurt should keep for a week or longer if held at 45^{o} F, or lower (normal refrigerator temperature). However, the population of viable bacteria present will experience a sharp decline within a day of two.

Apparent Health Benefits of Cultured Foods

Although studies conducted to test the health benefits of consuming cultured foods do not appear to yield consistent results, there have been a number of well-designed studies that indicate certain cultured foods and/or the organisms associated with them can have significant health benefits to a least some people. These benefits can be categorized into three areas including effect on lactose intolerance, effect on serum cholesterol, and anticancer effects.

Some degree of lactose intolerance is very common in adult humans. The condition manifests itself whenever certain quantities of milk or ice cream are consumed. The symptoms, usually including intestinal discomfort (due to gas) and diarrhea, occur because of a lack of the enzyme **lactase**. Without this enzyme to degrade lactose, intestinal microorganisms have more lactose available for their use, and consequently produce excess amounts of gas. Milk-products that have been fermented by bacteria prior to consumption contain reduced amounts of lactose, and so are less likely to cause the disagreeable effects. In some cases, microbial populations associated with cultured food products can live within the gut and so shift the overall gut community toward being homofermentative. Since homofermentative microbes produce lactic acid rather than gas, there is less gas produced overall.

Some studies indicate that yogurt contains factors that decrease the synthesis of cholesterol within the bloodstream. Other studies suggest that the bacteria associated with yogurt and acidophilus milk tend to remove cholesterol or its precursors from the gastrointestinal tract before it can be absorbed into the bloodstream. Thus it appears that the consumption of yogurt or other cultured food products may help reduce serum cholesterol levels.

The anti-cancer effects of yogurt have been demonstrated in a number of experiments involving non-human animals. These findings suggest that the consumption of cultured foods may be significant in reducing the risk of colon cancer.

Some Important Foodborne Pathogens

People involved in the preparation of food materials for immediate human consumption or potential long term storage with the intent of later consumption must take microorganisms into account. The hazards presented by foodborne pathogens vary depending on how and where foods are grown, how they are harvested, how long and under what conditions they are stored, and the circumstances surrounding their preparation. Though a complete list of foodborne pathogens is beyond the scope of this class, it is important for students to become familiar with some of the most commonly encountered examples.

Clostridium botulinum – Members of the genus *Clostridium* are obligately anaerobic endospore-formers. These pose a hazard when vegetable materials and meats are improperly canned or bottled. Viable spores germinating within food preparations can form cells capable of producing potentially lethal doses of the **botulism toxins**. These can cause potentially fatal flaccid paralysis.

Escherichia coli – Members of the genus *Escherichia* are facultatively anaerobic inhabitants of human and animal guts. They are a common cause of diarrhea, and in the case of O:157-H:7 can cause potentially fatal hemolytic uremic syndrome. These organisms have been found as contaminants in ground meat, spinach, fruit juices and other food materials.

Salmonella enterica – Various serotypes of *S. enterica* are known to cause typhoid fever (serotype *Typhi*) and gastroenteritis (serotype *Typhimurium*). Typhoid is spread through fecal contamination of food or water, and gastroenteritis is commonly associated with contaminated meat, milk and eggs. Infection with either strain can result in fatality.

Vibrio cholerae* & *V. paraheamolyticus – Bacteria in the genus *Vibrio* are common inhabitants of water, and in the case of *Vibrio cholerae* cause the potentially fatal disease **cholera**. Other *Vibrio* species, including *V. parahaemolyticus* cause gastroenteritis when ingested along with contaminated seafoods including fish, shrimp, crab, oysters and clams.

Staphylococcus aureus – Various members of the genus *Staphylococcus* inhabit human skin and nasal passages, so are readily transferred to food materials during preparation. When allowed to reproduce in foods, *Staphylococcus aureus* produces a heat-stable enterotoxin capable of causing severe episodes of vomiting and diarrhea.

Flukes, tapeworms, viruses and prions – **Fluke larvae** inhabiting water or fresh vegetable material can infect humans when ingested, as can **tapeworm larvae** found in various types of meat and seafoods. **Noroviruses** known as **Norwalk** or **Norwalk-like viruses** are a common cause of viral gastroenteritis. **Prions** are infectious proteins sometimes present in meats including beef, lamb and venison.

Questions:

1. What is rennet, and what is it used for in the production of cheese?
2. What are curds and whey? What is casein?
3. What is the difference between a ripened and an unripened cheese?
4. What types of bacteria are characteristically present in commercially produced yogurt?
5. What bacterial products give yogurt its characteristic flavor and aroma?
6. What are the apparent health benefits of consuming cultured foods?
7. Which food-borne bacteria can cause infection, and which can cause intoxication?

The Microbiological Table of Cheese

(Adapted from *Scientific American*, Sept. 1981)

CHEESE	ORIGIN	MICROORGANISM
	SOFT, UNRIPENED	
Cottage	Central Europe?	*Lactococcus lactis* and *Leuconostoc citrovorum*
Cream	United states	*Lactococcus cremoris*
Neufchatel	France	*Streptococcus diacetilactis*
	SOFT, RIPENED	
Brie	France	*Lactococcus lactis* & *cremoris* *Brevibacterium linens* and *Penicillium camemberti* or *candidum*
Camembert	France	*Lactococcus lactis* & *cremoris* *Penicillium camemberti* or *candidum*
Limburger	Belgium	*Lactococcus lactis* & *cremoris* *Breviibacterium linens*
	SEMISOFT, RIPENED	
Asiago	Italy	*Lactococcus lactis & cremoris* and *Streptococcus thermophilus* *Lactobacillus delbrueckii bulgaricus*
Blue (Roquefort)	France	*Lactococcus lactis* & *cremoris* *Penicillium roqueforti* or *glaucum*
Brick	United States	*Lactococcus lactis* & *cremoris* *Brevibacterium linens*
Gorgonzola	Italy	*Lactococcus lactis* & *cremoris* *Penicillium roqueforti* or *glaucum*
Monterey Jack	United States	*Lactococcus lactis* & *cremoris*
Muenster	Germany	*Lactococcus lactis* & *cremoris* *Brevibacterium linens*
	HARD, RIPENED	

Cheddar	Britain	*Lactococcus lactis, cremoris* and *Strep. durans, Lactobacillus casei*
Colby	United States	*Lactococcus lactis, cremoris* and *Strep. durans, Lactobacillus casei*
Edam	Netherlands	*Lactococcus lactis* and *cremoris*
Gouda	Netherlands	*Lactococcus lactis* and *cremoris*
Gruyere	Switzerland	*Lactococcus lactis, Strep. thermophilus* *Lactobacillus helveticus* and *d. bulgaricus* *Propionibacterium freudenreichii*
Stilton	Britain	*Lactococcus lactis* and *cremoris* *Penicillium roqueforti* or *glaucum*
Swiss	Switzerland	*Lactococcus lactis* and *thermophilus* *Lactobacillus helveticus* and *d. bulgaricus* *Propionibacterium freudenreichii*
	VERY HARD, RIPENED	
Parmesan	Italy	*Lactococcus lactis, cremoris* and *Streptococcus thermophilus* *Lactobacillus delbrueckii bulgaricus*
Romano	Italy	*Streptococcus thermophilus* *Lactobacillus delbrueckii bulgaricus*
	PASTA FILATA (PLASTIC CURD)	
Mozzarella	Italy	*Streptococcus thermophilus* *Lactobacillus delbrueckii bulgaricus*
Provolone	Italy	*Lactobacillus delbrueckii bulgaricus*

Name_____ **Lab Section**_____

WORKSHEET
Exercise 12
Food Microbiology – Fermented & Cultured Foods

Goals: _____

Materials & Methods:
Exercise 12A - Fermentation

Sauerkraut
Date: _____ Weight of cabbage: _____ x 0.03 = _____ (weight of salt to add)

Apple Wine
Date: _____ Juice used: _____

Exercise 12B – Cultured Foods

Microbial Flora of Cheese and Other Foods
Date: _____ Medium used: _____
Food products used: _____
Incubation temperature:_____ Duration of incubation:_____

Data & Results:
Exercise 12A
Sauerkraut

Date	pH	Date	pH

Sauerkraut Tasting (Only taste if there is NO mold. If there is mold, say so here.):
Date: _____ Description: _____
_____

Apple Wine

Initial °Bx: _____ (measured) Projected % alcohol: _____ (from table)

Date	Flask Weight (g)	Date	Flask Weight (g)

***NOTE**: Be sure the gas trap has the same amount of water for EACH READING.*

Total flask weight loss (g): _____

Apple Wine Tasting (Only taste if there is NO mold. If there is mold, say so here.):
Date: _____ Description: _____
_____

Exercise 12B
Microbial Flora of Cheese and Other Foods

Food Product	Color of Medium

Exercise 12A & 12B - Root beer & Cheese Tasting Notes

Root Beer "Vintage"	Carbonation	Aroma	Flavor

Cheese	Texture	Aroma	Flavor
Soft, Ripened Cheese (ie: Brie, Camembert)			
Semisoft, Ripened Cheese (ie: Blue, Monterey Jack, Asiago, Muenster)			
Hard, Ripened Cheese (ie: Cheddar, Gouda, Swiss, Colby)			
Very Hard, Ripened Cheese (ie: Parmesan, Romano)			
Plastic Curd Cheese (ie: Mozzarella, Provolone)			
Other Fermented Food (ie: yogurt, kefir, buttermilk, chocolate)			

Conclusions

The initial °Brix reading indicates the projected alcohol concentration that may be produced during the apple wine fermentation. Why is this only an estimate of alcohol production? (Why might the fermentation not reach this level?) _____
_____
_____

Why did the apple wine flask change weight over the course of the fermentation? _____
_____
_____

Based on the data obtained from the BCP-lactose plate, what do you know about the physiology of the organisms you cultured from yogurt and buttermilk? _____
_____
_____

Reflect upon your cheese, root beer, sauerkraut, and apple wine tasting experiences.
_____
_____
_____
_____
_____
_____