

Lecture 24

I. Revisit to Water Microbiology

A. Multiple Tube Fermentation Method (MTFM)

Confirmed Test

MacConkey/MUG



1. Select a fluorescent colony that ferments lactose (bright pink). Use this colony to inoculate a lactose broth tube (the _____).

2. Select a second, lac⁺ colony and use it to inoculate the _____. Choose either a fluorescent colony (presumed fecal) or a colony that does not fluoresce (presumed non-fecal).

II. Soil Microbiology

A. Isolation of differential types of soil microorganisms

1. Count the colonies on the plates used to select for growth of molds, *Streptomyces* species and other bacteria.

Knowledge check: Only plates containing between what two numbers of colonies should be counted?

2. Calculate the number of organisms per gram of soil:

i. Terms to remember

a. Titer: the number of organisms per milliliter

b. Concentration: The ratio of mass to volume of solution

Titer*(1/Soil concentration) or (organisms / mL)*(mL/g of soil) = number of organisms/g of soil

Knowledge check: Does the number recorded above represent the actual concentration of microorganisms in soil? Why or why not?

B. Isolation of nitrogen-fixing bacteria

1. A nitrogen-free medium _____ contain an inorganic source of nitrogen. Therefore, organisms that can live on this medium must be able to utilize _____.
2. Take a heavy inoculum of the nitrogen-free broth and _____.
3. The major genus represented on this plate will be _____.
 - i. *Azotobacter* are nitrogen fixers that are found in most _____.
 - ii. They are large, _____ that have the ability to form resting structures called _____.

III. Food microbiology

A. Food _____

1. Species of _____, *Erwinia*, and many lactic acid bacteria are common spoilage bacteria.
2. While spoilage bacteria lead to distasteful, smelly food, they are generally _____. However, a large number of spoilage microorganisms can indicate the presence of a _____.

B. Foodborne _____

1. _____
 - i. Results from the ingestion of a pathogen-produced _____ that contaminates food.
 - ii. *Staphylococcus aureus* produces an exotoxin that is an _____. It causes _____.
 - iii. *Clostridium botulinum* produces an exotoxin that is a _____ and can cause the _____.
2. _____
 - i. Requires the consumption of the _____. Ingested bacteria will grow, invade, and often secrete toxins. This leads to _____.
 - ii. Bacteria such as _____, and *Escherichia coli* 0157:H7 cause food infections.



C. Antimicrobial substances that are found naturally in food

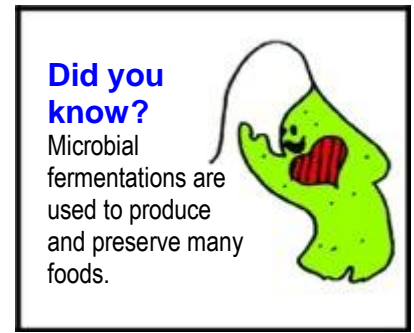
1. Food product: _____
 - i. _____ targets bacterial membranes
 - ii. Lactoperoxidase enacts _____
- 2 Food product: _____
 - i. _____ targets peptidoglycan
3. Food product: _____ (cloves, allspice, oregano, rosemary, sage, and vanilla)
 - ii. _____ denature proteins and perturb membranes
4. Food product: _____
 - i. Thiosulfates inhibit nucleic acid and protein synthesis
 - ii. _____ is thought to inhibit bacterial metabolism.
5. Food product: _____
 - i. _____ are thought to inhibit DNA gyrase, damage membranes, and inhibit catabolism.



III. Food production: _____

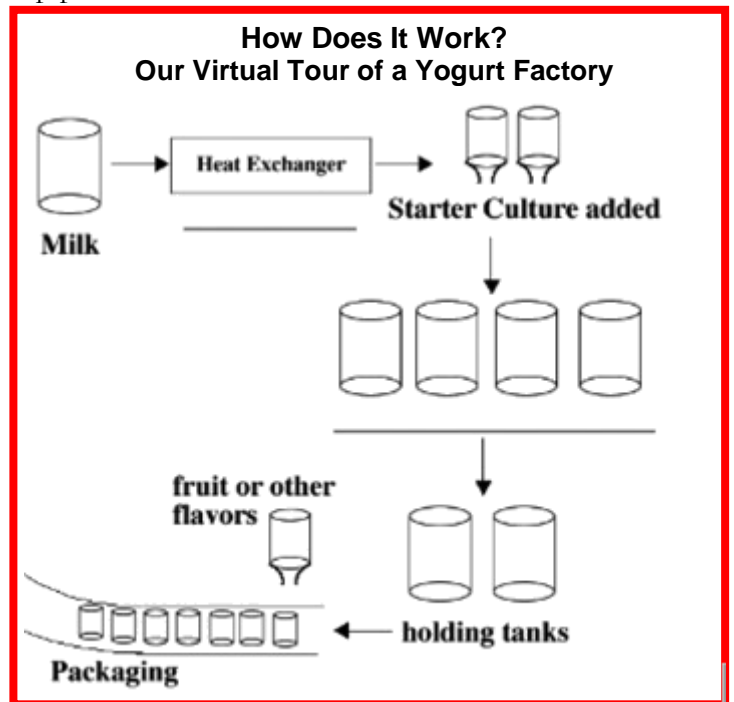
A. Dairy fermentations

1. Provide a way to _____, while at the same time providing products with various _____.
2. Used to produce _____, buttermilk, sour cream, and _____.
 - i. The starter culture of cheddar cheese is a mixture of _____ spp. The starter culture of _____ is a mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.
3. Performed by the _____
 - i. Include species in the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus*.
 - ii. These bacteria ferment the milk sugar _____ to lactic acid.
 - iii. The lactic acid production lowers the pH and _____.
 - iv. The lactic acid bacteria, also known as LAB, produce small antimicrobial peptides called _____. These bacteriocins help the LAB compete in the food medium on which they grow. Some of the bacteriocins are being used commercially to help preserve food.



4. Yogurt
 - i. One of the _____ fermented milk product in the U.S.³
 - ii. Produced both commercially and by individuals (yogurt-making kits).
 - iii. Produced using culture of _____

- a. *S. thermophilus* grows faster and renders the milk _____.
- b. *L. bulgaricus* further _____ the milk. The acidity causes the _____ and form a semi-solid curd.
- c. Eventually, the two species ferment all the lactose to lactic acid. They also produce _____ (*S. thermophilus*) and _____ (*L. bulgaricus*) that _____.



5. History
 - i. As long as 1,000 years ago, people began learning methods to _____.
 - ii. These “sour” milks have varied from country to country and have used as their source animals from _____.
 - a. The Russian kumis is made from _____.
 - b. The Swedish surmjÖlk comes from _____.
 - c. _____ is made from cow, goat, or sheep milk and originated in the Caucasus Mountains. Like kumis, it may contain up to 2% alcohol. Originally, kefir was produced in leather sacs and hung by the door. People passing by would massage the sack and stimulate fermentation.²
 - iii. For many years, the _____ was unknown. The initial microbial inoculation came from _____ used to make the products. Successive batches were inoculated with _____.

IV. _____

A. Live cultures in fermented milk products may provide health benefits.⁴

1. Control of diarrhea
2. _____ effects (some *Lactobacilli* have antitumor compounds in their cell walls)
3. Improvement of inflammatory bowel disease
4. Help maintain the normal balance of microbes in the gut and improve _____
5. _____

V. Prebiotics

A. "A non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improving host health."⁵

B. Examples include inulin, fructooligosaccharides, and transgalactooligosaccharides (plant-derived fiber). These are found in foods like _____, onions, _____, shallots, _____, sweet potatoes, _____, and others.

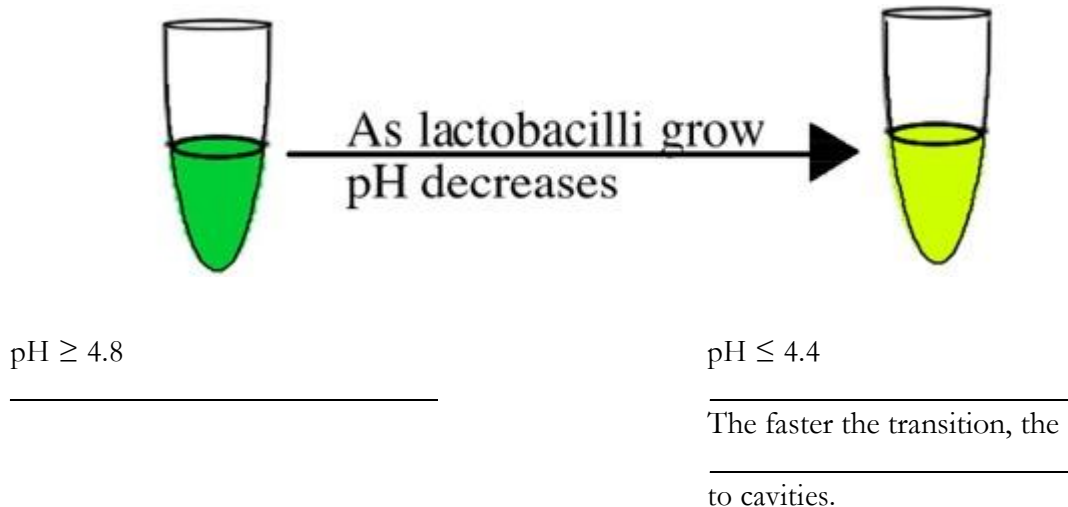
VI. Gut microbiota health is linked to _____, immune function⁶, _____⁷, and even mental health (depression and anxiety).⁸

VII. In today's experiment...

A. We will test *E. coli*, *S. aureus*, and *P. aeruginosa* for their susceptibility to a variety of natural antimicrobials found in food.

B. Caries susceptibility test

1. This test correlates the _____ present in the saliva to an individual's susceptibility to _____.



1. The Microbiology of Fermented Foods by Brian J.B. Wood

2. Prescott text (seventh edition) p. 1040

3. Prescott text (seventh edition) p. 1038

4. Prescott text (seventh edition) p. 1039

5. *International Journal of Current Microbiology and Applied Sciences*, Vol. 3 (2014) pp. 412PNAS (2014) Vol 111, no. 2

6. G.E. Mullin et al. (eds.), *Integrative Weight Management: A Guide for Clinicians, Nutrition and Health*. (ch. 7)

7. *Current Opinion in Biotechnology* 2015, 32:35-41