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

   $\text{Titer} \times \left( \frac{1}{\text{Soil concentration}} \right) = \frac{\text{organisms}}{\text{g of soil}}$

   **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 thermophilis 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 ________.

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

   ![As lactobacilli grow, pH decreases](image)
   
   pH ≥ 4.8
   ______________________
   pH ≤ 4.4
   ______________________
   The faster the transition, the ____________ to cavities.

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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
6. PNAS (2014) Vol 111, no. 2
7. *Current Opinion in Biotechnology* 2015, 32:35-41