EXPERIMENT 9

BACTERIOLOGICAL ANALYSIS OF HAMBURGER

Foods contain a variety of microbes that are either naturally occurring on the food item or are introduced in the processing of that food. The bacteria present can represent both a public health hazard as well as lead to the spoilage of the food. In this experiment we will determine the total number of viable bacteria as well as specific types of bacteria in samples of raw hamburger. The hamburger samples are weighed out and blended with sterile dilution fluid. Blending of food samples is common practice in the analysis of solid foods. The action of the blender renders the food homogenous and dislodges bacteria from the particles. **Bacteria are not harmed by blending**.

MATERIALS

1. Blender jars that are either autoclavable and sterile, or regular jars with 95% Ethanol to sterilize the jars and 500 ml of sterile distilled water/blender jar to wash out the alcohol.
2. 450 ml sterile dilution fluid (0.1% peptone) in 1 liter flask.
3. 99 ml sterile dilution fluid in a square bottle.
4. 2 - 9 ml dilution blanks (16x150 mm capped tubes).
5. Sterile 1 ml pipettes.
6. Balance, weighing boats, and spatulas.
7. 6 Tryptone-Glucose-Yeast Extract Agar plates (TGY).
8. 2 EMB Agar plates.
9. 6 Mannitol Salt Agar plates.
10. 6 Skim Milk Agar plates.
11. Fresh hamburger samples from different markets.

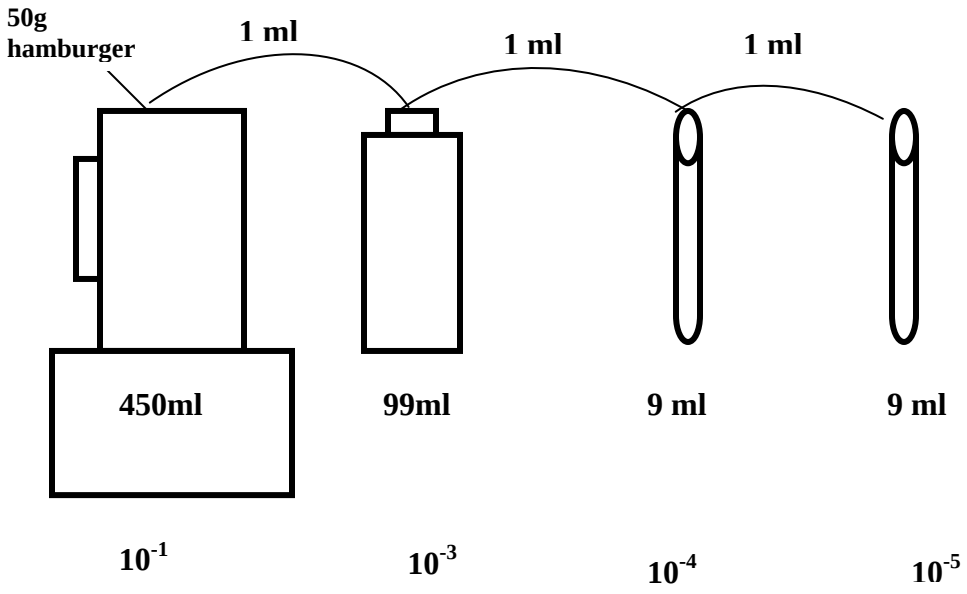
PROCEDURE

1. Weigh 50 grams of hamburger and add it to a sterile blender jar.

2. Add 450 ml of sterile dilution fluid and blend the sample for one to two minutes or until it appears thoroughly blended.

3. Allow the solids to settle and pipette one ml of the blended hamburger into the 99 ml dilution blank. Take care to get blended fluid only. The pipette can get clogged by the lipids which float to the top and the solids at the bottom; therefore, pipette from the middle of the blended hamburger.

4. From the now 100 ml dilution make two, serial 1:10 dilutions using the 9 ml dilution blanks. See diagram:



5. After the dilutions have been made, plate 0.1 ml of the 1:10 dilution (from the blender jar) onto each of two EMB plates. Use the spread plate method: spread the inoculum evenly over the surface of the plates with an alcohol sterilized (and flamed) glass rod.

6. Then plate 0.1 ml each of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions **in duplicate** onto Nutrient Agar, Mannitol Salt Agar, and Skim Milk Agar. Use the spread plate technique.

7. Incubate the plates at 37^{o}C for 48 hours.

8. After incubation, count the number of colonies on all the Nutrient Agar plates and calculate the total viable titer. Count the green sheen colonies on EMB, the yellow halo colonies on Mannitol Salt Agar, and the colonies with clear zones on the Skim Milk Agar. Each of these counts indicates the number of fecal coliforms, staphylococci, and proteolytic bacteria respectively.

RESULTS

HAMBURGER SOURCE:_____

Plate Counts

Nutrient Agar

Dilution	Colonies/Plate
10^{-3}	_____ , _____
10^{-4}	_____ , _____
10^{-5}	_____ , _____

Total Viable Titer:

_____ bacteria/gram

Mannitol Salt Agar

Dilution	Total Colonies/Plate	Yellow Colonies/Plate
10^{-3}	_____ , _____	_____ , _____
10^{-4}	_____ , _____	_____ , _____
10^{-5}	_____ , _____	_____ , _____

Total Halophile Titer:

_____ bacteria/gram

***Staphylococcus* titer:**

_____ *Staphylococcus*/gram

EMB Agar:

Dilution	Green Sheen Colonies/Plate	
1:10	_____ , _____	Coliform Titer: _____

Skim Milk Agar:

Dilution	Proteolytic Colonies/Plate
10^{-3}	_____ , _____
10^{-4}	_____ , _____
10^{-5}	_____ , _____

Proteolytic Bacterial Titer:_____

QUESTIONS

1. The USPH standards for hamburger are from 2.5 x 10^5 to 10^7 bacteria per gram. Hamburger is considered unsafe when the viable titer exceeds these standards. Was your hamburger safe?

2. Are the samples free of potential pathogens? Relate the total titer to the titers of coliforms and staphylococci.

3. What indication does the clearing colony titer from the Skim Milk Agar give to the shelf-life of the hamburger? What would a higher titer indicate?