EXPERIMENT 9

Identification and Quantification of Microbes in Food

INTRODUCTION

In recent days, the foods that we consume are usually pre-processed in a facility removed from our home, cities, countries, and even continents. It is now more than ever important to be aware not only what we eat, but also where our food comes from as many foods can be contaminated. **Contaminated** foods are those that possess high titers of infectious bacteria or have microbial products that are toxic to humans. Rules and regulations set forth by the Food and Drug Administration (FDA) are aimed at keeping potential food-borne illnesses at a minimum.

Although high standards in food preparation are the goals for public health, food-born bacterial contaminants are increasingly becoming a threat to public health. Thoroughly cooking processed meats and poultry is vital in order to provide a safer meal. Common infectious bacteria involved in the contamination of food include *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, and *Shigella* species.

In this lab, we will determine the presence of contaminating bacteria in food products. The ideal product we use in the lab is ground meat. Ground meat is especially susceptible to contamination since it is processed, therefore increasing the chances of encountering these microorganisms. Furthermore, when the meat id ground, at times other animal body parts including skin and fecal matter may be introduced and contaminate the food.

The purpose of this lab is to identify and enumerate the number of different bacteria in varying meat samples that can cause food illnesses and spoilage. In order to properly enumerate the number of contaminating bacteria, in this experiment we will be using serial dilutions and direct plating. A **serial dilution** is a stepwise dilution of a bacterial suspension through a series of tubes with sterile media. Every dilution is dependent on the previous dilution and the final dilution is the product of all the dilutions. The resulting plates from each dilution step will give a different number of colony forming units (CFUs). This dilution is made in a way to produce **countable plates** or plates

that have a range of 20-300 CFUs. Figure 1 indicates the serial dilution we will be performing in throughout this experiment (Figure 1).

In order to get a clear picture of the handling process and cleanliness of food products, we will be using a series of various agar mediums directed towards the growth of different bacteria including **Nutrient agar**, **Mannitol Salt Agar, Skim Milk Agar**, and **EMB plates**. Each media will shed light on the handling process of the food item.

To count the **total number of bacteria** found in the meat sample, we will be using the complex media Nutrient agar. This viable titer will indicate if the meat is safe for consumption, as a titer higher than $2.5X10^5$ to 10^7 bacteria per gram in considered unsafe for consumption.

A viable titer of the halotolerant/halophilic bacteria growing on the Mannitol Salt Agar plates indicates if the food item was properly handled and if any skin, hair, or mucosal passages have made contact.

The presence of E. coli growing on EMB plates will allow us to enumerate the fecal matter contamination in our food products. In order for safe food consumption, the E. coli titer must be zero.

Lastly, we will be using a Skim Milk Agar in order to observe the presence of Proteolytic Bacteria. **Proteolytic bacteria** are those that can break down meat protein and their presence indicates the degree of spoilage or shelf life of the meat sample. The larger the viable titer, the higher the degree of spoilage, and the lower the shelf life.

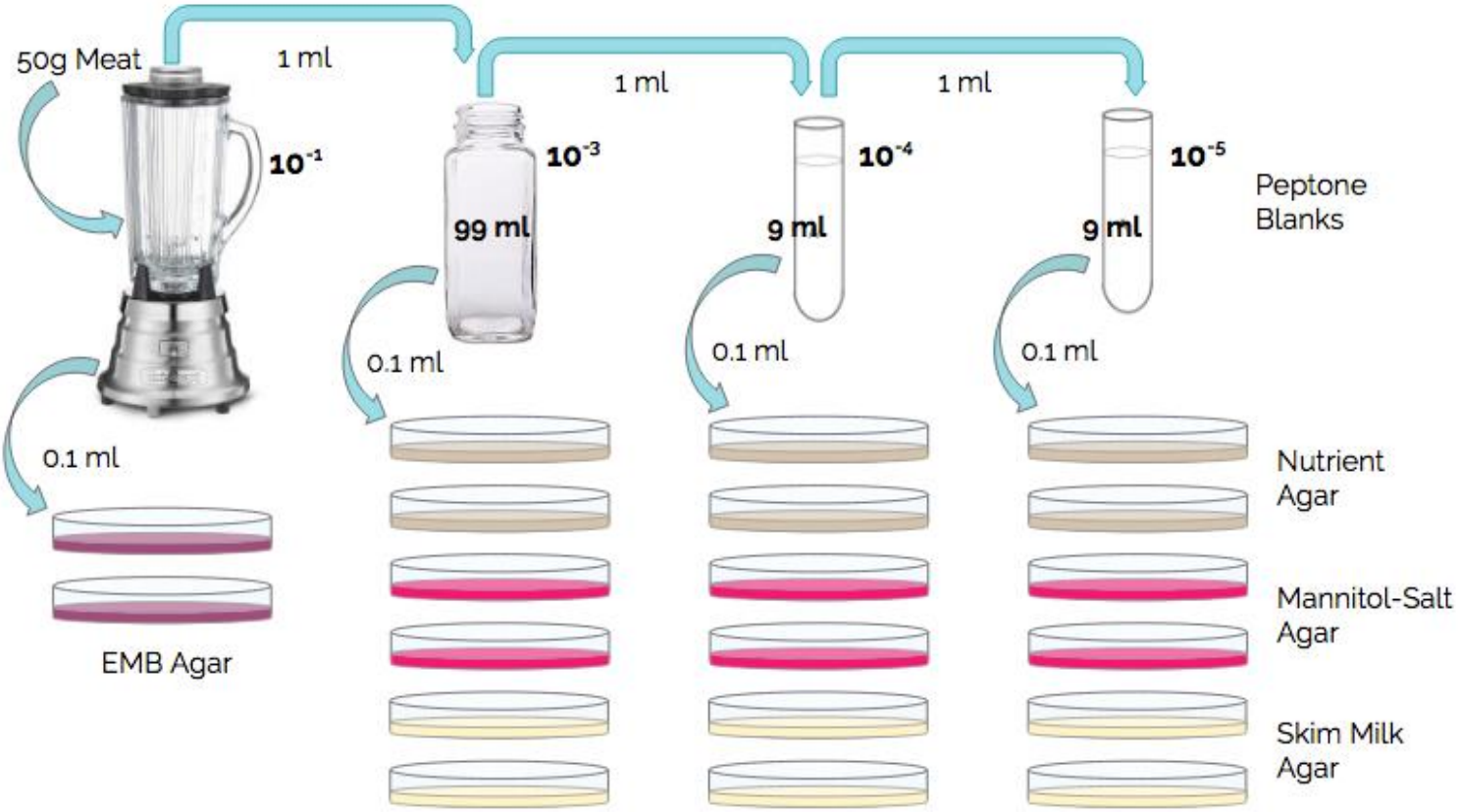

Figure 1. Serial dilution performed in this experiment in order to properly enumerate the bacteria titer from the meat sample.

Materials:

1. **Blender Jars with 95% ethanol for sterilization**
2. **500ml of sterile distilled water to rinse alcohol**
3. **450ml sterile dilution fluid in 1L bottle (0.1% peptone)**
4. **99ml sterile dilution fluid in square bottle**
5. **(2) 9ml dilution blanks (16x150mm capped tubes)**
6. **Sterile 1ml pipets**
7. **50g of pre-weighted ground meat**
8. **(6) Nutrient Agar plates**
9. **(2) EMB agar plates**
10. **(6) Mannitol Salt Agar plates**
11. **(6) Skim Milk Agar plates**
12. **Metal/Glass Spreader in beaker with 95% ethanol**

Procedures for WEEK 1:

1. LABEL all of your plates and tubes with your group designation, medium type, and the dilution that plate will be receiving.

2. Pulse the blender a few times with the 95% ethanol inside. Toss the ethanol in the appropriate container and use the 500ml sterile distilled water to remove the alcohol traces by pulsing the blender with water.

3. Discard the water in the blender down the drain. Pour the 450ml sterile dilution into the blender.

4. Obtain 50g of ground meat and add it to the sterile blender and blend it for one or two minutes until thoroughly blended.

5. After the solids have settled, pipette one ml of the blended meat into the 99ml dilution blank (Making a 10^{-3} dilution). The pipette will get clogged, so attempt to pipette near the middle of the solution.

6. From the 10^{-3} solution you just made, make two serial 1:10 dilutions using the 9ml dilution blanks. Refer to diagram in Figure 1.

7. After properly diluting in the blanks, inoculate 0.1 ml of the 10^{-1} dilution (from the blender jar) onto two EMB plates. Use the spread method to evenly cover the surface of the plate with an alcohol sterilized glass/metal rod.

8. Continue and inoculate 0.1ml of each of the remaining dilutions in duplicates onto each of the Nutrient Agar, Mannitol Salt Agar, Skim Milk Agar using the spread plate technique as mentioned above.

9. Incubate all the plates at 37°C for 48 hours.

Procedures for WEEK 2:

1. After incubation, obtain all of your plates and count all of the colonies as best as you can and calculate the viable titer for each organism type.

2. Count the total bacteria on the Nutrient Agar plates.

3. Count the green sheen colonies on the EMB plates.

4. Count the yellow colonies on the MSA plates.

5. Count the colonies with clear zones on the Skim Milk agar.

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REPORT 9

Part I. Plate Viable Counts

Directions: Record your results for the number of colonies counted from each pair of plates from each type of medium.

Plate	Dilution	CFU plate 1	CFU plate 2	Average
Nutrient Agar	10^{-3}			
	10^{-4}			
	10^{-5}			
	Total Viable Titer		**Bacteria/gram**	

Plate	Dilution	CFU plate 1	CFU plate 2	Average
Skim Milk Agar	10^{-3}			
	10^{-4}			
	10^{-5}			
	Proteolytic Bacteria Titer		**Bacteria/gram**	

Plate	Dilution	CFU plate 1	CFU plate 2	Average
EMB Agar	10^{-1}			
	Coliform Titer	**Bacteria/gram**		

Plate	Dilution	Total Colonies		Average	Yellow Colonies		Average
Mannitol Salt Agar	10^{-3}						
	10^{-4}						
	10^{-5}						
	Total Halophile	**Bacteria/gram**					
	Staphylococcus	**Staphylococcus/gram**					

Part II. Questions

1. According to the safety standards set forth by the USPH, is the hamburger you tested considered safe for consumption?

2. Is your hamburger sample free of potential pathogens that could come from fecal or skin sources?

3. What do the clearing colonies on a skim milk agar indicate about the shelf life of the hamburger? What would a higher titer mean?

4. Do you expect a piece of poultry or beef meat weighing the same to contain the same amount of bacteria as ground meat? Why or why not?

5. Which bacterial titer was the highest among the selective and differential media used? What does this tell you about your meat sample?