EXPERIMENT 6 ENUMERATION OF BACTERIA BY DILUTION AND PLATING

Structure

6.1 INTRODUCTION

The ability of microorganisms to grow and reproduce in food products is well known. Microorganisms may cause spoilage of the food product. Because of their very small size, counting the number of bacteria in a food sample can be difficult. Although direct counts are possible with a microscope, they require a lot of time and expertise. An easier method is to spread bacteria over a wide area (i.e. nutrient agar plate) and count the number of colonies that grow. If the bacteria are spread out enough, each bacterial cell in the original sample should produce a single colony. Usually, bacterial samples must be diluted considerably to obtain reasonable counts.

Objectives

After studying and performing this experiment, you should be able to:

- enumerate bacteria in food samples; and
- isolate pure colonies of bacteria.

6.2 EXPERIMENT

6.2.1 Principle

Since bacterial cell numbers are usually very high in your original sample, plating out this sample in an undiluted fashion would just lead to the creation of a bacterial lawn (a smear of many, many individual bacteria colonies that are all growing next to or on top of one another). Bacterial cell numbers need to be reduced, which is done by repeatedly diluting the amount of bacteria you have in your sample. A small amount of bacteria sample is mixed with a diluent solution (such sterile water or nutrient broth), and then successive dilutions are made. A small amount of each of the diluted bacteria samples is then spread onto an agar plate. The numbers of bacteria colonies that grow on each plate are counted. By working backwards using multiplication with the "dilution factor" (the number of times that you have diluted the bacteria sample with the diluent solution), you will be able to make a determination of the numbers of bacteria in your original sample.

For example, 10,000,000 cell per ml diluted to 100 cells per ml. It is virtually impossible to count 10,000,000 cells on the surface of the agar. However, it is much easier if we dilute the sample and only have to count 300 cells. Diluting is performed in increments

because we must obtain a plate with between 30 and 300 colonies (for statistical purposes). Figure 6.1 shows how isolated colonies should look like.

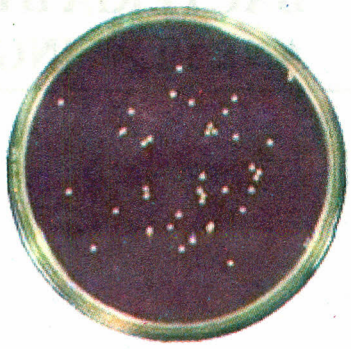

Figure 6.1: Bacterial Colonies Separated by Spread Plate Method

6.2.2 Requirements

- Nutrient agar plates (6)
- Large sterile tubes (2)
- Tubes with 9 ml of sterile nutrient broth (11).
- Sterile transfer pipettes
- Sterile sticks (2)
- Micropipettor
- Bacteria spreader
- 70% alcohol
- Food samples (say Sample A has been stored in a refrigerator for 4 days;
- Sample B has been stored frozen)

6.2.3 Procedure

DAY 1

1. Label 9 dilution broth tubes as follows:

   A 10^{-2}, A 10^{-3}, A 10^{-4}, A 10^{-5}, A 10^{-6}, A 10^{-7},

   B 10^{-2}, B 10^{-3}, B 10^{-4}

2. Label 6 agar plates as follows:

   A 10^{-6}, A 10^{-7}, A 10^{-8}

   B 10^{-3}, B 10^{-4}, B 10^{-5}

3. Label one large sterile tube A and the other large sterile tube B. Weigh aseptically 1 gram of sample A, and place it in sterile tube A. Add the contents of one tube of dilution broth to the food sample, and shake the sample until the suspension appears fairly uniform. Repeat this with sample B and sterile tube B.

4. Serial Dilutions

   Precaution: You must use a new sterile pipette for each of the dilution steps.

   Use a sterile pipette to transfer 1 ml of the suspension from large tube A to the culture tube labeled A 10^{-2}. Mix the contents thoroughly by pipeting up and

down several times. Use a new pipette to transfer 1 ml from tube A 10^{-2} to tube A 10^{-3} and mix thoroughly as before. Continue this series of dilutions into tubes A 10^{-4}, A 10^{-5}, A 10^{-6} and A 10^{-7}.

Repeat this series of dilution using sample B. Transfer 1 ml of suspension from large tube B into tube B 10^{-2} and mix thoroughly. Serially transfer, as before, into tubes B 10^{-3} and B 10^{-4}.

5. Plating bacteria

   Use a micropipettor to withdraw 0.1 ml of liquid from tube A 10^{-5} and place it onto the surface of the agar plate labeled A 10^{-6}. (NOTE: Plating 0.1 ml of a 10^{-5} dilution will give you the same number of colonies as plating 1 ml of a 10^{-6} dilution; the agar plate cannot absorb 1 ml of liquid, so the smaller volume is used.)

   Sterilize the bacterial spreader by dipping it into a beaker of alcohol. Remove and shake off the excess. Carefully run the spreader through the flame of a Bunsen burner and allow the alcohol to burn off. Cool the spreader by holding it against the condensation on the inside of the petri dish lid. Gently spread the liquid culture onto the surface of the agar by moving the spreader in a circular manner while rotating the plate. This will ensure an even distribution of bacteria.

6. Repeat step 5 with the remainder of the A cultures:

   Spread 0.1 ml from culture tube A 10^{-6} onto plate A 10^{-7}
   Spread 0.1 ml from culture tube A 10^{-7} onto plate A 10^{-8}

7. Repeat step 5 with the B cultures:

   Spread 0.1 ml from culture tube B 10^{-2} onto plate B 10^{-3}
   Spread 0.1 ml from culture tube B 10^{-3} onto plate B 10^{-4}
   Spread 0.1 ml from culture tube B 10^{-4} onto plate B 10^{-5}

8. Allow plates to absorb the cultures, then turn plates upside-down and incubate overnight at 37° C.

Precautions

1. Before plating, be sure to label each plate with its dilution, date and "food".
2. Mix the samples thoroughly before plating.
3. After pipetting the correct amount of sample in each plate, spread the sample with a bactispreader evenly over the entire surface of the agar.
4. Remember to use aseptic technique. Invert plates. Incubate at 37°C.

6.2.4 Observations

On DAY 2, after incubating the plates, count the colonies on the plate. Each colony represents one cell initially plated. For statistical purposes, pick a plate with between 30 and 300 colonies.

PLATE # COLONIES	PLATE # COLONIES
A 10^{-6}	B 10^{-3}
A 10^{-7}	B 10^{-4}
A 10^{-8}	B 10^{-5}
Bacterial count in sample A (refrigerated food):	Bacterial count in sample B (food):

6.2.5 Calculations

Determine the number of cells/gram in the original sample of food by multiplying the number of colonies on a plate by the dilution factor of that plate.

Calculations dilutions made	X	amount inoculated	=	"plated dilution"
dilution factor (simply the inverse of the plated dilution)	X	# colonies	=	# CFUs/ml (or gram) of the original undiluted sample

Example

If a plate labelled 10^{-7} has 87 colonies, then the sample has $87\times10^{7} = 8.7\times10^{8}$ colonies per gram.

6.2.6 Result

Find the average number of cells/g by adding the results from all of your plates and dividing by the number of plates.