EXPERIMENT 9 ENUMERATION OF MICROBES IN FOOD SAMPLES

Structure

9.0 OBJECTIVES

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

- prepare serial dilution of the sample for plating;
- enumerate bacteria in food samples;
- isolate pure colonies of bacteria; and
- perform total viable count.

9.1 INTRODUCTION

The microorganisms grow and reproduce in food products because food is a good source of the nutrients required for their growth. 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. 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. For estimation of microbiological load, a serial dilution is made. The idea of serial dilution is to reduce the concentration of organism in successive manner so that distinct visible countable colony could be produce on media plates. The usual serial dilution of 1:10 may be prepared using 1 ml innoculum and 9 ml diluting solution. In case of food samples 1:10 serial dilution is prepared.

9.2 PREPARATION OF SERIAL DILUTIONS

In order to enumerate the microorganisms in a meat sample, a pre-measured quantity (90 ml) of a suitable sterile diluent is first added to a meat sample (10 g) taken in a sterile conical flask (250 ml). The contents are homogenized in a sterile stomacher/blender for 2 minutes to make 1: 10 dilution of the sample (label the flask as 10^{-1}). Further serial ten fold dilutions of this diluted sample (1:10) are made by serial addition of each dilution

(1 ml) to the sterile diluent (9 ml). Label 6 dilution broth tubes as follows:

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

You must use a new sterile test tube and a sterile pipette for each of the dilution steps. Transfer 1 ml of the suspension from flask (10^{-1}) to the tube labeled 0^{-2}. Mix the contents thoroughly by pipetting up and down several times. Use a new pipette to transfer 1 ml from tube 10^{-2} to tube 10^{-3} and mix thoroughly as before. Continue this series of dilutions into tubes 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

Diluents: The most commonly used diluents for preparing serial 10-fold dilutions of a meat samples are:

a) Peptone water (0.1%)

b) Normal saline solution (0.85 %)

c) Phosphate buffered saline

9.3 TOTAL VIABLE COUNT (TVC)

One of the most common tests carried out to judge the microbiological quality of foods is the Total Viable Count (TVC), which is also known as 'Aerobic Plate Count' (APC). To avoid lab-to-lab variations in the results of microbiological analysis of food samples, the uniformity with regard to media, incubation conditions and test protocols of TVC to be used in different labs have been standardized, which is called as 'Standard Plate Count' (SPC). In this method, suitable dilutions of a food sample are plated on or in an agar-based medium called SPC agar containing complex nutrients such as beef extract, yeast extract and peptone (a proteolytic enzyme digest of fresh meat containing a variety of inorganic salts, growth factors and peptides) to support the growth of wide range of microorganisms. The pH of the SPC agar is usually adjusted to 7.0 to 7.4 so that bacteria rather than yeasts or moulds are recovered.

The agar plates are seeded (inoculated) with sample dilution containing the bacterial cells and placed (incubated) at suitable temperature in an incubator. Thus, an estimate of the total number of viable cells in the dilution plated out can be calculated by counting the total number of bacterial colonies which develop following incubation.

The incubation temperatures selected depend on the food being examined. Commonly used temperatures are 55°C for thermophiles, 35 to 37°C for mesophiles and 20°C for many spoilage bacteria. Whilst the latter temperature is suitable for psychrotrophic bacteria as well as for many mesophiles, lower temperatures (e.g. 1-7°C) are sometimes used for more accurate estimates of psychrotrophs; it must always be borne in mind that no one incubation temperature completely excludes all organisms from another group. Many plating techniques are employed for enumerating total numbers of viable bacteria and three are described briefly. Enumeration of colonies is traditionally performed manually using an illuminated colony counter with the operator counting each individual colony. This can be a tedious operation and, unless a suitable number of colonies has developed in the growth medium (ideally between 30 to 300) it can also be inaccurate. In recent years, automatic colony counting devices have been developed which enable accurate counts within a few seconds.

Note: Sabouraud's Dextrose Agar (SDA) is used in place of SPC agar for examining the yeast and moulds.

9.3.1 Requirements

- Nutrient agar
- Large sterile tubes
- Tubes with 9 ml of sterile nutrient broth
- Sterile transfer pipettes
- Sterile sticks
- Micropipette/pipette
- Bacteria spreader
- 70% alcohol
- Food samples

9.3.2 Procedure

1) Label tubes with 9 ml diluents in each as follows:

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

   Take one tube with 1gm meat and 9ml diluent or one flask with 10 gm meat with 90 ml diluent and label as 10^{-1}. Follow the method described under preparation of serial dilution.

2) Label plates as follows:

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

3) Plating and incubating: Any one of the following method can be used for plating.

Pour Plate Method

In this method, a set (or preferably triplicate sets) of Petridishes is inoculated with 1 ml aliquots from appropriate dilutions of the food. (e.g. inoculate 1ml of sample from a tube containing 10^{-2} dilution to a plate marked as 10^{-2}). Approx. 15-20 ml of molten SPC agar, cooled to 45°C, is then added to each of the Petridishes. Petridishes are covered with their lids. Move plate gently five times clockwise and five times anticlockwise on the table to ensure proper mixing of agar and sample. After the agar has solidified, the plates are incubated at the required temperature for a period depending upon the incubation conditions (e.g. 1-2 days at 37°C, 3-4 days at 20°C and 7-10 days at 5°C).

Spread Plate Method

Here the medium is pre-poured and allowed to solidify in the Petridishes; 0.1 ml of the dilutions are spread evenly over the whole surface of the medium using sterile L-shaped glass rods. Here 0.1ml sample from tube 10^{-2} spread on media of plate 10^{-3}. Follow the same trend for rest of the plates. Plates are incubated as above. Advantages of this technique are, firstly, that heat-sensitive cells (i.e. psychrotrophs) are not killed by the molten agar which may occur to an extent in the 'pour plate' method if the temperature of the agar is too high; Second advantage is that all the colonies develop on the surface of the agar and can be easily observed and picked off if necessary, whereas, many colonies develop embedded in the agar in the 'pour plate' method, and these may be restricted in size and may be more difficult to subculture. The rest of the procedure and interpretation of result is same as in case of pour plate technique.

Drop Plate Method

Solidified medium is again used with this technique. Specially calibrated pipettes delivering 0.02 ml per drop are used and five separate drops (i.e. 0.1 ml) are delivered onto the surface of the plate, the drops being dried before incubation. Dilutions giving under 20 colonies per drop should be counted.

9.3.3 Observation

After incubation, plates containing between 30-300 colonies should be selected for counting the colonies on them and an average of colonies on three Petridishes for that particular dilution is calculated. The number of viable cells per gram (or per cm^2 if sample was taken from per cm^2 area of meat surface by swab) of food can then be readily calculated.

9.3.4 Result

If an average count of 112 colonies is obtained for the 10^{-4} dilution, the total viable count per gram of food would be $112 \times 10^4 = 1.1 \times 10^6$. Since each colony represents a bacterium, the result should be expressed as Colony Forming Units (CFU) per gram of food sample.

In other words, CFU/g = Average of no. of colonies x Reciprocal of the sample dilution.

9.4 PRECAUTIONS

- Every glassware and equipment should be sterilized before use.
- While preparing serial dilution mix well the contents and for transfer from one tube to another a new sterile pipette should be used. Never use a pipette that has come in contact with higher dilution for transfer to lower dilution.
- While putting for incubation the petridishes must be kept in inverted position.
- While putting the mark on Petridishes it should be preferably on upper lid so that colony visibility could not get hindered.
- Before plating, be sure to label each petridish with its dilution and if more than one sample is used then write the date and food no. also.