

Enumeration of microorganisms in foods

Introduction

The raw, semi-processed and processed foods as well as the ingredients used in the preparation of processed foods may contain several microorganisms. The microbial examination of foods is generally done to know the presence or absence of specific microorganisms, types of microorganisms, number of microorganisms and their products in foods. The information on the microorganisms associated with food will help in;

- ✚ Estimating shelf life (suitability for human consumption) by determining microbiological quality of a food or constituent of food.
- ✚ Identifying the cause of spoilage or presence of pathogens, when food is implicated in food borne illness.

Methods of enumeration

Several methods are available for the enumeration of microorganisms from food. These can be broadly divided in to direct methods or indirect enumeration methods based on whether the microorganisms are counted directly or the products released by them is estimated.

I. Direct enumeration methods

a. Direct counting methods

- ✚ Direct microscopic count (DMC)
- ✚ Direct counting on membrane filters

b. Culture based methods

Plate count method

- Pour plate method
- Spread plate method

Most probable number (MPN) technique

II. Indirect enumeration methods

a. Alternative Methods (chemical and physical methods)

- Dye reduction test
- Electrical methods

- ATP determination

b. Rapid Methods

- Immunological methods
- DNA/RNA methodology

Study of microorganisms in foods by conventional methods

Enumeration of microorganisms in foods is traditionally performed by directly counting all microorganisms present in foods or by allowing them to develop in to colonies and then counting.

Direct counting methods

Microorganisms present in the food can be counted by observing the food sample directly or retaining the microorganisms on a filter paper by filtering the sample and then observing under microscope.

A. Direct microscopic count (DMC)

DMC involves detecting the presence of microorganisms in food by microscopic observation. It is a simple method and easy to perform.

DMC is performed by making a smear of food specimen/cultures on to microscopic slides, staining with appropriate dye and viewing and counting all cells using microscope under oil immersion objective. This method can be used only when microorganisms are present in large numbers (10⁶/ml). It is commonly used in dairy industry for assessing microbial quality of raw milk and other dairy products.

Advantages

- Rapid and easy enumeration
- Can be employed to any foods (Ex: dried/frozen foods)
- Simple to perform
- Cell morphology can be assessed
- Efficiency can be increased by using florescent probes

Disadvantages

- Requires tiresome counting under microscope causing fatigue to analyst
- Counts both viable and non-viable cells
- Food particles may interfere with counting and mistaken for microorganisms
- Cells may not be distributed uniformly (single cells/ clumps)
- Some cells may not take up stain and missed while counting
- DMC counts are always higher than standard plate count
- Requires dilution of sample

B. Direct counting on membrane filters

Membrane filters with pore size smaller (0.45 μm) than bacteria retain bacteria and the retained bacteria can be counted using microscope.

Procedure involved

- ✚ Concentrating/collecting bacteria on polycarbonate filters by filtering known volume of homogenized sample.
- ✚ Staining and counting of retained bacteria.
- ✚ Placing the membrane on a nutrient agar media or absorbent pad saturated with culture media of choice, and incubating
- ✚ Following growth, colonies are counted

Advantages

- ✚ Well suited for samples containing low numbers of bacteria
- ✚ Facilities concentrating bacteria by filtering large volume of sample
- ✚ Only small volume of food samples can be used for a single membrane
- ✚ Efficiency of membrane filter method can be increased by staining with fluorescent dyes (Ex. acridine orange) and observing under epifluorescence microscope (DEFT: Direct Epifluorescence Filter technique). Viable cells fluoresce green and are counted. Non viable cells appear orange. Acridine orange is a metachromatic fluorochrome which binds to double stranded DNA of viable bacterial cells.
- ✚ Can be used to enumerate microorganisms from a variety of foods (fresh fish, meat, fish/meat products, water samples etc).

Culture based methods

Culture methods involve examination of microorganisms in food by encouraging them to multiply in a liquid or solid media. On solid agar media bacteria develop as colonies and counting such viable colonies gives microbial load in foods.

Enumerating microorganisms by culture based methods can be done by using plate count methods or MPN technique.

Culture media

A wide variety of media with varied composition capable of supporting the growth are available for the cultivation of different microorganisms.

The composition of the media varies depending on;

- ✚ Group/type of microorganism to be studied
- ✚ Overall purpose of the study
- ✚ Whether to grow wide range of microorganisms or specific types

- ✚ Resuscitation of damaged but viable cells
- ✚ Type of diagnostic information required

Ex: General purpose media: Plate count agar

Lactose broth: For *Escherichia coli*

Selective media: Baird parker agar for *Staphylococcus*

Bismuth sulphite agar for *Salmonella*

TCBS for *Vibrios*

A. Plate count method

This method is variously referred to as total plate count (TPC), standard plate count (SPC) or aerobic plate count (APC). It is most widely used conventional method for determining viable cells or colony forming units (CFU) in foods.

SPC involves blending/ homogenizing the sample, serially diluting in appropriate diluent, plating in or on suitable agar media, incubating at appropriate temperature for a given time, and counting visible colonies as CFU.

Principle involved

SPC is based on the principle that each viable bacterial cells multiples and grows in to a visible colony. Thus, counting number of colonies gives an idea about bacterial cells present in a sample. Counts determined by taking average of replicate plates showing 30-300 colonies.

Factors affecting SPC

- ✚ Sampling method employed.
- ✚ Distribution of microorganisms in food.
- ✚ Nature of food biota
- ✚ Nutritional adequacy of plating media
- ✚ Incubation temperature and time
- ✚ Type of diluent used
- ✚ Presence of other competing organisms etc.
- ✚ Plating on selective media for specific organisms is limited by degree of inhibition and effectiveness of selective/ differential agents employed.

SPC can be performed by pour plate method or spread plate method (surface plating method)

a). Pour plate method

Appropriate dilution of the sample (1 ml) is mixed with agar medium, allowed to set, incubated at appropriate temperature and colonies developed are counted. Here colonies develop both on surface and subsurface of agar plate.

Proper mixing of sample with agar medium is necessary so as to get isolated colonies which can be done by 2 ways.

- ❖ One ml of appropriate sample dilution is added to petri-plate and about 15 ml of agar medium is added and mixed by rotating the plate in clockwise and anticlockwise direction.
- ❖ One ml of appropriate sample dilution is added to test tube containing about 15 ml of molten agar medium, mixed by rolling the tube between the palm and poured to petri-plates, allowed to set and incubated.

b). Spread plate method

Diluted sample (0.1 ml) is spread on the surface of pre-poured, hardened agar plates using glass rod, incubated at appropriate temperature and colony developing on surface counted.

Advantages

- Suitable for heat sensitive psychrotrophs in food as they do not come in contact with molten agar.
- Enables providing colony features useful in presumptive identification especially on selective media.
- Favours strict aerobes on surface, but microaerophils grow slowly

Disadvantage

- Problem of spreaders and colony crowding makes the enumeration difficult.

B. Most probable number (MPN) technique

MPN is suited for enumerating the presence of low numbers of microorganisms in foods. This involves inoculating replicate tubes of appropriate liquid media (3 or 5 tube) with three different sample sizes/ dilutions of the material to be studied and incubating at appropriate temperature. Then the absence or presence of growth is observed and MPN table consulted to get probable number of organisms in the sample. MPN numbers are generally higher than SPC.

Advantages

- Relatively a simple method and assay to perform
- Results are comparable from one laboratory to another
- Specific group of organism determined by use of specific media.
- Suitable for detecting organisms present in low numbers
- Method of choice for coliform detection

Disadvantages

- Requires use of large number of glassware and large volume of sample
- Can not observe colony morphology
- Lack of precision