UNIT 5 GENERAL TECHNIQUES OF DETECTION AND ENUMERATION OF MICRO-ORGANISMS IN FOOD

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5.0 OBJECTIVES

After reading this Unit, you will be able to:

- identify various types of microbiological media and their method of preparation;
- describe different enumeration techniques of micro-organisms;
- prepare pure culture of micro-organisms from mixed population; and
- identify micro-organisms on the basis of microscopic observation.

5.1 INRODUCTION

Food microbiology is the branch of microbiology that deals with various types of micro-organisms occurring in the food products. These range from the beneficial micro-organisms to the harmful ones that contaminate foods and lead to its spoilage or disease out breaks by utilizing food as a vector for their spread causing food borne illness. Micro organisms may be added deliberately in the preparation of food products and in this case they are called the starter cultures that play an important role in determining the taste, texture and other properties of a food product. Various types of fermented milk products like dahi, yoghurt, cheese, kefir, kumiss etc. are prepared by addition of particular starter cultures to the milk from different sources. These cultures bring about the required changes in the milk such as lactose utilization and at times may also provide health benefits to the consumer. These benefits may be due to the changes carried out by microbes such as lactose utilization, which alleviates the symptoms of lactose intolerance or microbes may have a more direct effect when consumed in live active forms. In the latter case, these micro-organisms

are called 'Probiotics' that have been linked to several health benefits. Probiotics have a positive effect on individual's health only when they are consumed in required numbers. Whether we talk about probiotics, spoilage or disease causing microbes, the detection and quantification of the microbes in the food product is very important as it points towards the quality of the food product. In case of probiotics, the functionality of the food product depends upon the cell numbers of specific probiotic strain in the food product. However, in the case of food spoilage the overall bacterial load should be taken in account. Apart from probiotics or spoilage causing microbes another category is that of pathogenic micro-organisms occurring in food products. With the production of food products at industrial scale the presence of pathogens in food can cause food borne out break of a food borne disease. So the detection of pathogens in food products is of prime importance to prevent such out breaks.

Keeping all these facts in view, the contents of this book have been formulated and organized to incorporate the relevant methodologies employed in general techniques of microbiological analysis of foods, screening and enumeration of spoilage and pathogenic organisms and some rapid methods of microbiological analysis.

5.2 MICROBIOLOGICAL MEDIA

Each organism needs to find in its environment all the substances required for energy generation and cellular biosynthesis. Micro-organisms are known to thrive in almost every environment where they play an important role in the bio-geochemical cycles and maintenance of food chains. They grow in particular niche and consume the nutrients existing there, increase their numbers. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as nutrients or nutritional requirements. The concentration of nutrients varies in the environment and this controls various microbial populations. In the laboratory, micro-organisms are grown in culture media which are designed to provide all the essential nutrients in solution for their growth. Several types of media are known and the choice of media depends upon the specific microorganism to be grown. Though there are different kinds of media but there is similarity between the media used for a particular group of micro-organisms. Some media can support the growth of several microbes while others are specific. Common media used for growing bacteria is nutrient broth which contains beef extract (carbohydrates, organic nitrogen compounds, water soluble vitamins, salts), peptone (organic nitrogen) and yeast extract (B vitamins, organic nitrogen, carbon compounds).

5.2.1 Types of Media

As discussed in the above paragraph there are several hundreds of media and their modifications known for culturing bacteria. However, these media are characterized broadly into several types such as:

1. **Chemically defined media/ synthetic media** - In this type of media the exact chemical composition is known. It is used for the cultivation of autotrophs. Ex. Potato dextrose agar
2. **Complex media**; The exact chemical composition of this media is not known. It contains substances such as yeast extract, beef extract, peptones, brain heart infusion, hydrolysates, tissue extracts, blood serum etc. The exact composition of these raw materials added to the media is not known

Nutrient broth is an example of complex media. Such media are used for growing heterotrophs.

3. **Liquid media**: This type of media are also known as broths. Broths do not contain solidifying agents and are in liquid state. They are used for routine cultivation procedures for pure culture or mixed cultures. These media are also used for production of various compounds during industrial fermentations.
4. **Solid media**: When a solidifying agent is added to the liquid media it is called solid media. The most common solidifying agent used for media preparation is agar. It gets solidified below 45^{o}C and is usually added at a concentration range of 1.5-2.0%. It is inert and does not get degraded by most of the bacteria; however, a few bacteria are known to produce agarase, which play a role in the degradation of agar. Solid media is used for isolation of bacterial colonies by streaking or serial dilution methods. It is also helpful when colony characteristics of a bacterial culture are to be studied as in case of differential media. Nutrient agar is an example of solid media.
5. **Semi solid media**: This contains a low concentration of agar as compared to solid media. A gelly like structure is formed in this type of media. It is used for studying the motility of the bacterial cultures or for the cultivation of micro-aerophilic bacteria.
6. **Selective media:** This media allows the growth of only a particular type of bacteria from the mixed culture e.g. addition of antibiotics (say Penicillin) to the media renders it selective for the growth of only antibiotic resistant bacteria (Penicillin resistant strains). Selective media is used extensively for isolation of bacteria from the environmental samples and also for various screening methods in molecular biology experiments where a plasmid is carrying a drug resistance gene.
7. **Enrichment media**: This type of media is used for favouring a particular bacterial strain from the mixed culture. A particular compound which enhances the growth of the strain of interest is added to the media. This strain grows at an enhanced rate and soon dominates in the culture.
8. **Differential media**: This type of media is used for the differentiation of bacterial cultures by studying their growth and colony characteristics e.g. *E. coli* is known to form blue black colonies with a green metallic sheen when grown on Eosin Methylene Blue (EMB) agar. Thus this media can be used for differentiating *E. coli* from other bacteria.

5.2.2 Preparation of Media

Most of the bacteriological media used in microbiology are available as dehydrated powders. The required amount of powder is weighed and dissolved in distilled water. The pH of the media is adjusted as per instructions provided by manufacturer. Usually for routine cultivation of bacteria nutrient broth with pH 7.0 is used, while potato dextrose medium with pH 5.0 is used for fungi.

In preparation of nutrient media (broth/agar) pH is adjusted before autoclaving at 121^{o}C for 15 min, but in case of potato dextrose agar (PDA), this adjustment is done by addition of sterilized solution of 10% tartaric acid after autoclaving the media.

At times microbiological media contain heat labile components and in this case stock solutions of such components are filter-sterilized by passing them through a bacteria proof membrane filter having pore size ≤ 0.2µm under positive pressure separately and added to the remaining autoclaved media to obtain the desired concentration. There are several variations in the preparation of media but all the methods follow one basic scheme depicted in the following flow chart.

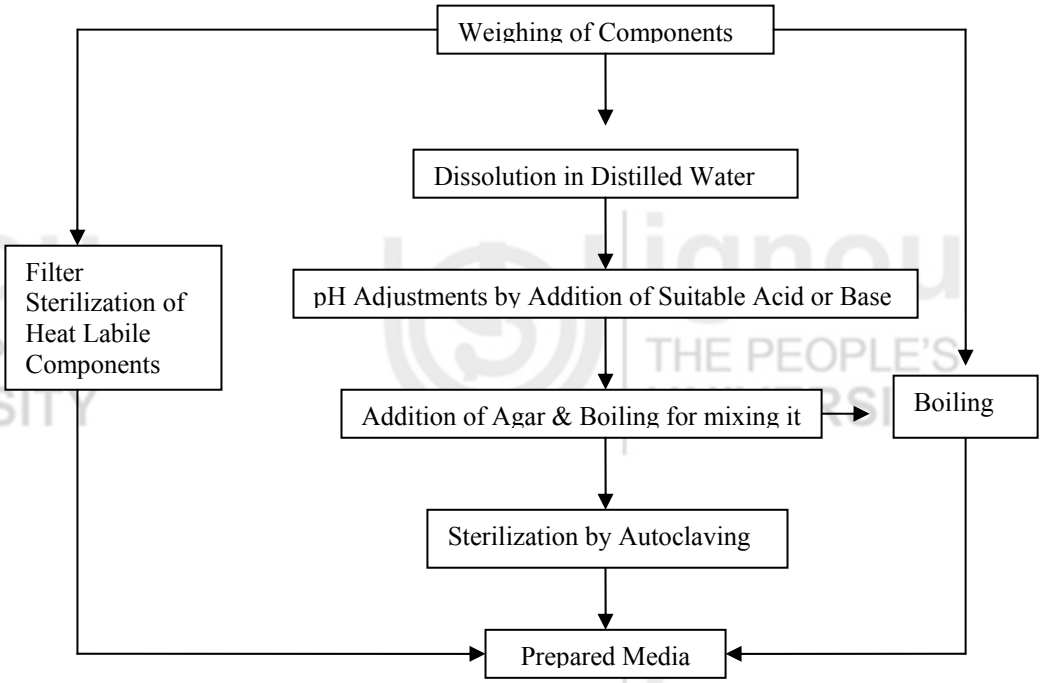

Fig 5.1: Preparation of Microbiological Media

The form in which media are prepared also vary e.g. solid media can be prepared in flasks and poured into plates whenever required e.g. for Standard Plate Count (SPC) or it may be prepared as slants, deep tubes or stabs for biochemical and maintenance purposes.

Care should be taken that the glassware used for media preparation is clean and plugged properly so as to avoid contamination. Use of freshly prepared media should be avoided and it should be incubated at ambient temperature to make sure that autoclaving is proper and there is no development of contaminants.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) Name some defined and complex media. What is the difference between them?

……

……

……

2) Differentiate between enriched, defined and differential media.

……

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3) Which is the solidifying medium for microbes?

……..

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4) What are probiotic micro-organisms?

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……..…

5.3 ENUMERATION PROCEDURES

Enumeration procedures for micro-organisms can be classified into direct methods where the numbers of micro-organisms are counted as in the case of Direct Microscopic Count (DMC), Standard Plate Count (SPC), Spiral plating or indirect methods where overall microbial load is correlated to the metabolic activity of the micro-organisms as in dye reduction tests.

5.3.1 Direct Microscopic Count (DMC)

Direct microscopic count is carried out to estimate the number of bacterial cells or the somatic cells in raw and processed milk. The direct microscopic count can be related to the quality of milk. Presence of a large number of bacterial and somatic cells in milk may point to the mastitic condition of the milching animal.

Also, if there is reason to suspect that a food has caused food poisoning or has undergone microbial spoilage, the original product or a low serial dilution of it should be used to prepare a slide for direct microscopic examination. The DMC of sample is carried out by examining stained smear of a measure volume of sample say milk (0.01 ml) spread over a known area of glass slide (1.0 cm^2) and counting the number of cells or clumps of cells in a microscopic field. The average number of cells per microscopic field is determined after staining 5 to 50 fields. The diameter of a field is measured with a stage micrometer for calculation of microscopic factor (MF). The total number of bacteria per ml is calculated by multiplying average number of cells with microscopic factor.

The method is simple and rapid and facilitates simultaneous enumeration and observation of cells. It can be conveniently used for screening and grading of foods especially, liquid foods. However, major disadvantage is that both dead as well as cells are taken into account and hence less suitable for heat treated foods.

The total number of bacteria as follows:

Total number of bacteria /ml= N x MF

where, N is average number of cells per field; and

MF stands for microscopic factor $\left\{ MF = \frac{A_s}{A_m} \times \frac{1}{V} \right\}$

As is area of smear (100 sq. mm)
AM isarea of microscopic field
V is volume of milk (0.01 ml)
DMC=10,000/3.1416 × r^2

(where, r is the radius of microscopic field as determined by using stage micrometer and 100X objective).

Precautions recommended during direct microscopic count

- Never dust a lens by blowing on it. Saliva will inevitably be deposited on lenses and is harmful, even in minute amounts. Never use facial tissues to clean lenses. They may contain glass filaments which can scratch lenses. Linen or chamois may be used for cleaning but may not be as convenient as lens tissue.
- Never leave microscope tubes open. Always keep them closed with dust plug, eyepiece, or objective, as appropriate.
- Avoid touching lenses. Even light fingerprints, especially on objectives, can seriously degrade image quality.
- Avoid getting immersion liquid on non-immersion objectives; it can damage the lens mounting glue.
- Use the following procedure to properly clean lenses of microscopes or other optical equipment. Crumple a piece of lens tissue to create many folds to trap dirt without grinding it into the lens. Do not touch the part of the tissue that will be applied to the lens; excessive touching transfers natural oils from the fingers to the lens tissue. Apply a small amount of lens cleaning solution to the lens tissue and blot the tissue against absorbent material to prevent fluid from entering the lens mount. Wipe the lens very lightly to remove gross dirt that was not blown away by the rubber bulb. If necessary, repeat the cleaning process with a new piece of lens tissue and with slightly more pressure to remove oily or greasy residue.

5.3.2 Standard Plate Count (SPC)

Standard Plate Count is carried out to estimate the number of viable bacteria in a food sample. Bacteria growing aerobically in the mesophilic range of temperature are detected in SPC. A high SPC of the food product indicates poor sanitary conditions of the food processing plant and a low SPC indicates the good quality of the processed food in terms of bacterial load, however, SPC does not indicate the absence of pathogens in the finished food product. SPC is based on the serial dilution of the food sample in sterilized dilution blanks followed by plating on the nutrient media. The dilution of sample is required because usually food samples contain a high number of bacteria, which are difficult to count. If undiluted sample is plated, a lawn will form on the plate and the individual isolated colonies that can be counted will not be formed. After serially diluting the representative sample of food, plating is carried out by pour plate or spread plate technique. In pour plate technique, the dilution is added to the sterile petriplate followed by the addition of molten nutrient agar. The diluent and agar are mixed and plates are incubated after solidification for the growth of bacterial colonies. Pour plate technique is the preferred technique for carrying out SPC, however at times, spread plate technique which involves the spreading of the dilution on the surface of solidified nutrient media may also be used. The SPC of the food sample is reported as colony forming units per ml (cfu/ml). The term colony forming unit is used because the bacterial colony obtained on the agar plate may not be produced by a single bacteria but bacterial clumps may also give rise to the colony hence cfu/ ml and not cells/ ml is used for reporting the results.

Calculation and Results

For calculating SPC only those plates having the colony numbers from 25 to 250 are considered. Plates having more than 250 colonies are designated as Too Numerous To Count (TNTC). Alternatively in such cases count may be reported as estimated count.

Number of colonies on valid plates (colonies ranging from 25-250) are counted. The average obtained from the duplicates at a single dilution is multiplied by the dilution factor to obtain the colony forming units per ml (cfu/ml) of the food sample e.g. if plates at 10^{-4} dilution are having 50 and 60 colonies the cfu/ml will be calculated as:

cfu/ml = average no. of colonies at a dilution x respective dilution factor

$$\left(\frac{50+60}{2}\right)\times 10^{4} = 5,\ 50000 = 55 \times 10^{4} = 5.5 \times 10^{5}$$

Precautions

1) The sample taken for analysis should be representative of the whole stock. If a sample taken for analysis is small it will give erroneous results.
2) Blending of the sample should be done carefully so as to prepare a homogeneous suspension.
3) Dilutions should be mixed properly before each transfer.
4) Molten agar should not be very hot (40-42^{O}C is optimum temperature) at the time of pouring.
5) Plates should be incubated in an inverted position so as to avoid moisture condensation and consequently lawn formation on the surface of agar.
6) Diluent taken may differ with the type of food product. Sodium citrate (2%) may be used for fatty foods such as cheese. Normal saline, buffers or even distilled water may also be used as diluent.

5.3.3 Spiral Plate Count

This method can be said to be a variation of Standard Plate Count. In this a very small volume of inoculum is plated on the surface of the plate and this gives a high dilution (10,000:1) of inoculum on a single plate. A mechanical device called Spiral Plating Machine is used for this purpose.

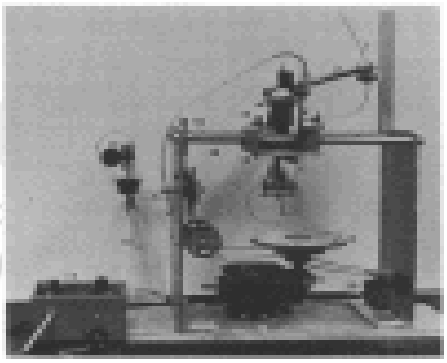

Fig 5.1: Spiral Plating Machine

It dispenses the inoculum on the surface of rotating hardened agar plate. The plate is then incubated at the suitable incubation temperature depending on the bacterial culture plated on the plate. After incubation the isolated colonies are counted using a counting grid that covers the area of agar plate and is divided into four concentric circles and eight wedges or octants.

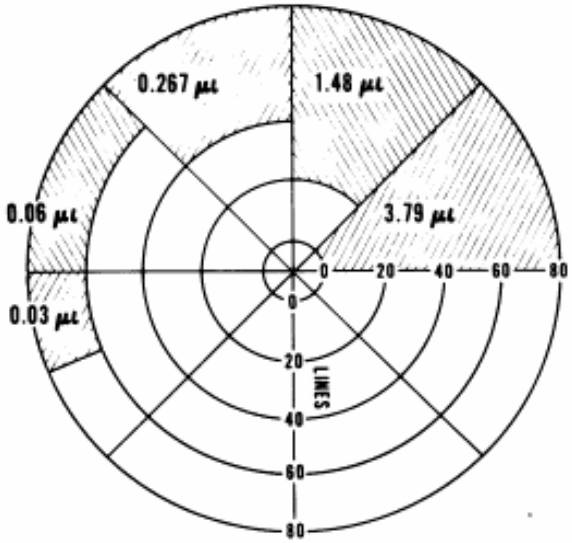

Fig 5.2: Spiral counting grid

The well separated isolated colonies are counted in the wedges and total number of cells present in the sample is calculated accordingly.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) Which of the counting methods is fastest:
   (i) DMC
   (ii) SPC
   (iii) Spiral counting
2) List a major drawback of DMC?

   ……

   ……

   ……
3) What is a mesophile?

   ……

   ……

   ……
4) The pouring temperature of agar in petriplates is ……
5) Write the formula of microscopic factor.

   ……

   ……

   ……

5.3.4 Dye Reduction Tests

These tests are used to determine the microbiological quality of milk. These are indirect tests where the number of micro-organisms is not counted as in SPC; instead the time required for reduction of dyes (methylene blue or Rezaurin) is

taken into account and correlated with the milk quality. These dyes act as oxidation-reduction (O-R) indicators and get decolorized due to the consumption of oxygen and decrease in the O-R potential of milk as a result of bacterial growth.

A. Methylene Blue Reduction (MBR) test

Methylene blue is a dye, which remains blue in its oxidized state and turns colourless on its reduction. This characteristic is put to use for estimation of bacterial load of milk and milk products. When bacteria grow in milk they release hydrogen during respiration, which is simultaneously accepted by methylene blue. As a result, it is reduced to colourless or leuco compound.

The majority of bacteria, both aerobic and facultative present in milk indulge in lowering of oxidation-reduction potential of milk to such an extent that dye gets decolorized. Hence greater the number of viable cells, shorter is the time taken to reduce the dye. The result of this test is expressed in terms of time required for the colour of methylene blue to disappear as Methylene Blue Reduction (MBR) time at incubation temperature of 37^{o}C.

This test renders very useful information on general bacteriological quality of milk in a short period and requires minimum apparatus. Limitations of this technique include its suitability only for unheated milk and no indication of the type of organisms. The methylene blue reduction time also depends on the type of bacteria occurring in milk. Coliforms reduce this dye at a higher rate as compared to psychrotrophic and thermoduric bacteria occurring in milk. This is so because the incubation temperature favours the growth of mesophilic bacteria (coliforms). High numbers of lecucocytes if present also affect reduction time of methylene blue in milk samples.

Requirements

Milk sample, methylene blue solution (0.4 mg/100 ml of sterile distilled water), sterile tubes and stoppers, sterile pipettes, water bath.

Procedure

1. Take 10 ml milk in a test tube and add 1 ml of methylene blue solution to it.
2. Insert stoppers on the tubes and mix the contents by inverting the test tubes gently.
3. Incubate the test tubes in a covered water bath at 37^{o}C and check the tubes for decolourization after every 30 min. of incubation.
4. Remove the decolorized tubes after each reading and also invert gently the remaining tubes.
5. Monitor the tubes for decolourization till 8 h.

Interpretation

The quality of milk is graded according to the time taken for decolorization and is as follows:

Class 1. Excellent, not decolorized in 8 h

Class 2. Good, decolorized in less than 8 h but not less than 6h.

Class 3. Fair, decolorized in less than 6 hours but not less than 2 h.

Class 4. Poor, decolorized in less than 2 h.

Precautions

- Dye solution should be prepared and kept in amber bottles without exposure to light.
- Milk should be mixed properly but gently because vigorous shaking will increase the oxygen content of milk. If mixing is not proper then bacteria may arise with the cream layer and this leads to inaccurate results.
- Water bath should be covered as exposure to light will decrease the methylene blue reduction time.

B. Resazurin Reduction (RR) test

Resazurin is also an O-R indicator and hence is liable to be reduced by bacteria. Reduction of blue dye takes place in two stages. First, the dye is irreversibly reduced to resorufin undergoing a series of color changes ranging from blue to lilac, mauve, purple and pink. During second stage, resorufin is reversibly reduced to a colourless compound, dihydroresorufin.

Various colors that develop in a sequence during reduction of dye can be well compared with a standard resazurin disc with the help of a small apparatus known as resazurin comparator. Results are expressed in terms of standard resazurin disc number ranging from 6 to 0. The time taken for the reduction of dye to a specific stage (disc number) or the color change recorded on completion of incubation after a certain period can be used as a scale for measurement of bacterial activity. The test is carried out in several formats including "one-hour" and "triple reading" tests.

This test finds its application in quick grading of milk (even faster than MBR test). However, reduction of dye is susceptible to light and confusion may arise in interpretation of results due to the fact that besides bacteria, this dye is also liable to be reduced by leucocytes.

Requirements

Milk sample, resazurin solution (11 mg/ 200 ml of hot sterile distilled water), sterile tubes and stoppers, sterile pipettes, water bath.

Procedure

1. Take 10 ml milk in a test tube and add 1 ml of resazurin solution to it.
2. Insert stoppers on the tubes and mix the contents by inverting the test tubes gently.
3. Incubate the test tubes in a covered water bath at 37^{o}C and check for the change in the colour after 1 hr of incubation in case of 'one-hour' test or at 1, 2 and 3 h for "triple reading" test.
4. Compare the colour with the standard resazurin disc number ranging from 6 to 0 and report the results.

5.3.5 Most Probable Number (MPN) Method

Most probable number is based on the probability of the presence of bacteria in standard dilution series of the sample inoculum. Bacteria within the dilution series are detected by positive growth characteristics such as gas production or turbidity in the growth medium. An MPN procedure just like SPC detects the viable organisms and is useful when bacteria are present at low concentration (< 100/g) in food, milk, water and soil samples. However, MPN gives only the

estimate of organisms while plate count gives direct counts in cfu/ ml. MPN is used to estimate the number of coliforms in the water samples, however, this method can be modified to estimate the number of pathogens by using specific selective culture medium for their growth. Sanitary analysis of the water sample employs the lactose broth medium with Durham's tube (a small inverted tube added to the larger tubes containing the lactose broth) that helps in the detection of gas production by coliforms from lactose utilization. This test is also called the presumptive test for coliform detection.

5.4 PURE CULTURE METHODS

The methods listed in the above sections are for determining the microbial load/ counts in the food samples; however, some of these methods such as pour/spread plating can also be used to get the pure cultures of bacteria. These methods have an advantage that they can be used both for obtaining bacterial counts and the purification of mixed cultures. Apart from these methods another method called streak plate can also be used for purification of microbial cultures. Streak plate method involves diluting the bacterial culture on the surface of agar medium so as to obtain isolated colonies of bacteria. These colonies are then differentiated on the basis of morphology (differential media) or other tests (biochemical). The main disadvantage of using this method is that you cannot count the number of bacteria as the dilution in this case is not equated. Streaking can be carried out in several formats such as continuous streaking, quadrant streaking, T streaking etc. but all involve the same principle of diluting the culture on the surface of agar plate so as to obtain isolated bacterial colonies.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) The period of observation of MBRT is …….. hours.

2) Streaking is done to …….

3) What is the principle behind resazurin test technique?

……

……

……

4) ……. broth is used in MPN technique.

5.5 MICROSCOPIC EXAMINATION OF THE BACTERIAL CULTURE

Bacterial cultures are examined under 100X oil immersion lens of the microscope. They may be examined as unstained cultures when the motility of the culture is to be studied or they may be stained depending upon the requirement. Several types of staining is carried out for studying bacterial culture including negative staining, grams staining, endospore staining, capsule staining, flagellar staining, acid fast staining etc.

5.5.1 Simple Staining

In this type of staining bacterial cells are stained with a single reagent. Positively charged dyes such as methylene blue, crystal violet etc. are used for this purpose. These stains are taken up by the cells and bind to negatively charged cell components (cell wall, nucleic acids).

5.5.2 Negative Staining

In this method the bacterial cells do not take up the stain but the background gets stained and the cell appears as unstained transparent entity. Acidic dyes such as nigrosin are used. Negative staining is advantageous because the bacteria which do not take up the stain can be observed in this way. Another advantage of this procedure is that it does not require heat fixing of smear so cell distortion due to heat do not take place.

5.5.3 Gram Staining

This is the most important staining technique for the bacterial cultures. Based on gram staining, bacteria are characterized into gram positive cells and gram negative cells depending on their capacity to retain crystal violet stain. The basis of gram staining is the difference in the cell wall structure of the two types of bacteria. Gram positive cell walls have a higher content of peptidoglycan and a lower content of lipids while gram negative cells have higher concentration of lipids and lower concentration of peptidoglycan. During gram staining, the bacterial smear is stained with crystal violet and iodine followed by destaining with alcohol. Lipids present in the cell wall of gram negative cells gets dissolved in alcohol forming pores through which crystal violet leaks out, while peptidoglycan in the cell wall of gram positive shrink due to alcohol, thus retaining the crystal violet and hence the purple colour.

5.5.4 Endospore Staining

Endospores are highly refractile, heat resistant bodies that are produced by certain bacteria when the conditions are not favourable for growth. The heat resistance of endospore is an important property and allows survival of bacteria in the form of metabolically inactive endospore, which germinates on return of favourable conditions and start growing, contaminating food and spoiling it. Endospores are stained with Malachite green dye under hot conditions. Afterwards the slide is stained with saffranine (pink), the other microbes appear pink while endospores which do not stain with saffranine appear green.

5.6 LET US SUM UP

For cultivation of micro-organisms in laboratory and their enumeration, suitable media are required to be prepared and these can be classified as general purpose, selective, differential, and enrichment on the basis of application. These media are used for enumeration of microorganism by various techniques namely, DMC, SPC, spiral plate count, dye reduction methods, and MPN method. The streak plate method can be used for isolation and purification of microorganism present in food. The micro-organisms so isolated and purified can be microscopically identified and morphologically studied on the basis of staining methods such as simple, negative, gram and endospore staining.

5.7 KEY WORDS

EMB	**:**	Eosin methylene blue
PDA	**:**	Potato dextrose agar
DMC	**:**	Direct microscopic count
MF	**:**	Microscopic factor
SPC	**:**	Standard plate count
cfu	**:**	Colony forming unit
TNTC	**:**	Too numerous to count
MBRT	**:**	Methylene blue reduction test
RRT	**:**	Resazurin reduction test

5.8 SUGGESTED FURTHER READING

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5.9 TERMINAL QUESTIONS

1) Define microbiological medium and describe their types.
2) Enlist various enumeration techniques and suggest their appropriate applications in various food systems.
3) How can you calculate microscopic factor?
4) How you can differentiate between gram positive and gram negative bacteria? What is the basis of gram reaction?

5) While performing Coliform count of water by MPN method, no of positive tubes in 10 ml, 1.0 ml and 0.1 ml are 2,1,0 respectively. Determine the count/ ml with the help of MPN table.

6) How can you observe flagellum of a bacterial cell? Describe the protocol of a suitable method.

5.10 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include following points:

1) Defined media: Potato Dextrose agar; Complex media: Ex. yeast extract, beef extract, peptones, brain heart infusion, hydrolysates, tissue extracts, blood serum etc. The exact composition of these raw materials added to the media is not known.

2) Selective media allows the growth of only a particular type of bacteria from the mixed culture and is used extensively for isolation of bacteria from the environmental samples and screening methods in molecular. Enrichment media is used for favouring a particular bacterial strain from the mixed culture. This strain grows at an enhanced rate and soon dominates in the culture. Differential media is used for the differentiation of bacterial cultures by studying their growth and colony characteristics.

3) Agar

4) Probiotic micro-organisms have been linked to several health benefits when consumed in live active forms.

Check Your Progress Exercise 2

Your answer should include following points:

1) DMC

2) Even dead microbes may be counted under microscope in DMC.

3) Mesophiles are the microbes which are able to grow at room temperature.

4) $MF = \frac{A_s}{A_m} \times \frac{1}{V}$

Check Your Progress Exercise 3

Your answer should include following points:

1) 8
2) isolate microbial colonies.
3) Resazurin is an O-R indicator and can be reduced by bacteria. Reduction of blue dye takes place in two stages. First, the dye is irreversibly reduced to resorufin undergoing a series of color changes ranging from blue to pink. During second stage, resorufin is reversibly reduced to a colourless compound, dihydroresorufin. The time taken for the reduction of dye to a specific stage (disc number) or the color change recorded on completion of incubation after a certain period can be used as a scale for measurement of bacterial activity.
4) Lactose