EXPERIMENT 1 PREPARATION OF MEDIA

Structure

1.1 INTRODUCTION

Microorganisms require certain basic nutrients and physical factors for the sustenance of life as do all other living organisms. However, their particular requirements may vary greatly. Nutritional needs of microbial cells are supplied in the laboratory through a variety of media to detect their presence. Microbiological media for the evaluation of spoilage and detection of bacteria, yeasts and molds in foods are mentioned in this chapter.

Objectives

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

- learn that microorganisms are ubiquitous and in nature, they do not segregate themselves by species but exist a mixture of many other cell types; and
- learn to make different media which are suitable for a particular genera of microorganisms to produce their discrete colonies.

1.2 EXPERIMENT

1.2.1 Principle

Many special purpose media are needed to facilitate recognition, enumeration and isolation of different types of microorganisms. To meet these needs, the microbiologists has developed numerous media which on the basis of their function may be classified as follows:

A) Selective Media

These media provide nutrients that enhance the growth and predominance of a particular type of bacterium and do not enhance (and may even inhibit) other types of organisms that may be present. For instance, a medium in which cellulose is the only carbon source, will specifically select for, or enrich the growth of cellulose-utilizing organisms when it is inoculated with a soil sample containing many kinds of bacteria.

B) Differential Media

Certain reagents or supplements, when incorporated into culture media, may allow differentiation of various kinds of microorganisms. For example, if a mixture of bacteria is inoculated on to a blood containing agar medium (blood agar) some of the bacteria may hemolyze (destroy) the red blood cells, others do not. Thus one can distinguish between hemolytic and non-hemolytic bacteria on the same media.

1.2.2 Requirements (Equipment/Machinery/Instrument/Chemicals/ Material)

- Autoclave (Portable)
- Balance
- Heating mantle/ water bath
- pH meter
- Laminar air flow
- Stirrer
- Pipettes
- Distilled water
- Media (nutrient agar, potato dextrose agar, violet red bile agar, plate count agar)
- Test tubes
- Beakers
- Cotton plugs
- pH paper
- Measuring cylinder

1.2.3 Procedure

The preparation of microbiological media usually involves the following steps:

1. Carefully weigh the proper amount of the dehydrated base medium or the correct proportion of constituent ingredients and dissolve in appropriate volume of distilled water and heat. Composition of the media is as given in Annexure-1.
2. Determine the pH of the medium, and adjust if necessary with dilute acid or alkali.
3. If a solid medium is desired, add agar (1.5-2%) and boil the medium to dissolve the agar.
4. Distribute the medium into tubes or flasks. The amount of medium distributed per container should be limited so that no point within the volume of the medium is more than 2.5 cm from the top surface of the container.
5. Autoclave at 121°C for 15 minutes. Some media (or specific ingredients) that are heat labile are sterilized by filtration.

1.2.4 Observations

Observe the pH using the pH meter or colour indicator solutions. At the next laboratory period observe for any contamination to ensure proper sterilization. Discard any contaminated flasks and wash them in disinfectant solution.

1.2.5 Result

After performing this experiment you will observe that suitable media for growth of bacteria is nutrient agar (pH 6.8-7.0) or plate count agar (pH 7.0), whereas for yeasts and moulds it is potato dextrose agar (pH 5.6) and malt agar (pH 5.4).

1.3 PRECAUTIONS

- Adjust the pH of the media accurately, to provide favourable condition of growth for the microorganisms. pH of the medium may change during sterilization and because of possible browning reactions, it is important not to exceed the recommended time and temperature.
- Prepare medium in such quantities that if stored, it will be used before loss of moisture through evaporation that becomes evident.
- To prevent contamination and excess evaporation of water from a medium in flask and tubes during storage, optionally fit aluminium foil or plastic with loose rubber bands before autoclaving in order to allow air to escape and to prevent the container from bursting.
- Avoid over loading autoclaves so that the rate of air exhaust and heating is not appreciably delayed. The autoclave should reach 121°C (15 psi) slowly but within 10 min. after starting the air exhaust operation.
- Flask or test tubes should be plugged with cotton or capped with paper.
- After sterilization gradually reduce the pressure within the autoclave (using no less than 15min) since liquids may be at a temperature above their boiling point at atmospheric pressure. If the pressure is lowered too rapidly, liquids may boil over and come out from the container.
- Used plates, pipettes, tubes etc. should be routinely decontaminated by autoclaving for 30 minute at 121°C.
- Media should be stored at 2-8°C in a dry, dust free area and should not be exposed to direct sunlight.