PRACTICAL 3 CULTURE MEDIA

Structure

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3.1 INTRODUCTION

Practical 3 deals with *culture media*. What do we mean by *culture media*? Culture media, you would realize, is *a solid or liquid preparation containing all the nutrients required by microbes for growth*.

So then, what are the nutritional requirements of microorganisms? You have already studied about these in the Food Microbiology and Safety theory Course in Unit 3. Look up the Unit now. Understanding the nutritional needs of the microorganisms is essential for its cultivation and maintenance in the laboratory. This practical focuses on this aspect and also the culturing and maintenance of microbes under laboratory conditions. What are the different media and how to prepare media is the highlight of this practical.

Objectives

After undertaking this practical and conducting the exercises given herewith, you will be able to:

- discuss the nutritional requirements of microorganisms for growth,
- describe the culturing and maintenance of microbes under laboratory condition,
- explain the media and its types, and
- prepare the different media for growth and maintenance of microorganisms.

3.2 NUTRITIONAL REQUIREMENTS OF MICROBES: AN INTRODUCTION

Microorganisms require various nutrients and physical factors for their existence and growth. Nutrients are substances used in biosynthesis and energy release for microbial growth. Larger quantities are required for some nutrients as these are the parts of various building blocks like, lipids, proteins, carbohydrates and nucleic acids. These are called *macronutrients*. Other elements are needed in very small amounts and are serving as co-factors or parts of the enzyme. These are called *trace elements*. Environmental factors such as temperature, oxygen level, pH and osmotic concentration of the medium are also critical for microbial growth, as we have already studied in the theory course in Unit 3. In this Practical, let us briefly recapitulate the nutritional needs of microorganisms.

About 95% of dry cell weight of microorganisms is constituted by macro elements. These are carbon (C), nitrogen (N), oxygen (O), hydrogen (H), sulphur (S), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe). In addition to macro elements, all microorganisms also require several microelements or trace elements. These are Iron (Fe), Manganese (Mn), Zinc (Zn), Cobalt (Co), Molybdenum (Mo), Nickel (Ni) and Copper (Cu). These are normally a part of enzyme cofactor and helps in catalysis of reactions and maintenance of protein structures, e.g. Mo^{++} is needed for nitrogen fixation, Co^{++} is a part of Vitamin B_{12} and so on. Generally, these are obtained as contaminants from glassware, water and other media components. Micronutrients are generally ubiquitous in nature and probably do not limit the growth. Besides, common macro and trace elements, some microorganisms may have special nutrient requirements like, diatoms require silicic acid, many bacteria require high concentration of sodium ions and so on.

Following components are essential for the growth of microorganisms:

1. *Carbon* – Carbon is needed for the skeleton of all organic molecules. Microorganisms acquire it either as inorganic carbon in the form of carbon dioxide (autotrophs) or from organic nutrients (heterotrophs). Microorganisms, in general, have extraordinary flexibility with respect to carbon sources. There is no naturally occurring organic molecule that cannot be used by some microorganisms. Some bacteria can use almost anything as carbon source while others are fastidious and employ only few carbon compounds.

2. *Nitrogen* – Nitrogen is an essential atom in certain cellular macromolecules, e.g. amino acids, purines, pyrimidines, enzymes, cofactors etc. Some microbes use atmospheric nitrogen, while others rely on inorganic nitrogen compounds as nitrate or ammonium salts. Still some others can use only organic compounds like amino acids.

3. *Sulphur* – Sulphur is a part of some amino acids, some carbohydrates, biotin and thiamine. It is obtained either as elemental sulphur, inorganic sulphur e.g., as SO_4^- or organic compounds like amino acids.

4. *Phosphorus* – Phosphorus is used in the form of phosphate salt by microorganisms. It is involved in formation of nucleic acids, phospholipids, several cofactors and energy rich compound ATP (adenosine triphosphate).

5. *Potassium, Calcium, Iron and Magnesium* – These are supplied by inorganic salts and exist in the cell as cations. These perform various functions in the cell like stabilizing ribosomes and cell membrane, needed for enzyme activity, giving heat resistance to endospores, or a part of cytochromes etc.

6. *Vitamins* – Vitamins are also essential in small amounts for cellular activities and growth. These are also the coenzymes for active enzyme systems. These usually make up all or part of the enzyme cofactor. Some microbes can synthesize vitamins while others need it from outside source.

7. *Water* – All cells require water for various cellular activities.

8. *Source of Energy* – Constant supply of energy is required to carry out cellular activities, like biosynthesis and degradation of macromolecules, transport etc. Two sources of energy are available for microorganisms -

    i) *Light Energy* – Organisms using light as a source of energy are called phototrophs.

ii) *Energy derived from organic or inorganic molecules* – Organisms using energy obtained by oxidation of organic or inorganic chemical compounds like glucose, H_2S etc. are called chemotrophs.

9. *Source of Electrons* – Depending upon the source of electrons used by microorganisms, these are known as -

   a) Lithotrophs (rock eaters) – use reduced inorganic substance as electron source.

   b) Organotrophs – use electrons from organic compounds.

Microorganisms require a balanced mixture of these elements. Nutritional requirement for different microorganisms vary and can be met through different ways. Microorganisms can be divided into different nutritional types based on their requirement. Let us in the next section see how the microorganisms are classified based on their nutritional requirements.

3.3 NUTRITIONAL TYPES OF MICROORGANISMS

Depending upon the nutritional requirement, microorganisms can be broadly placed into 4 nutritional types.

(1) *Photolithoautotrophs* – These microorganisms use light as an energy source, inorganic substance as electron source and CO_2 as the primary carbon source. Example: algae, purple and green S bacteria and Cyanobacteria.

(2) *Photoorganoheterotrophs* – These microorganisms use light as a source of energy and organic compound as electron and carbon source. Example: purple and green nonsulphur bacteria.

(3) *Chemolithoautotrophs* – These microorganisms use inorganic compound as source of energy and electron and CO_2 as a source of carbon. Example: sulphur oxidizing bacteria, hydrogen bacteria and nitrifying bacteria.

(4) *Chemoorganoheterotrophs* – These microorganisms use organic compound as a source of energy, electron and carbon. Example: protozoa, fungi and most non-photosynthetic bacteria.

Most of the microorganisms belong to photolithoautotrophs and chemoorganoheterotrophs. Though a particular species belongs to only one of the four categories, some species are *mixotrophs*, i.e., these alter their metabolic activities according to environmental conditions, e.g., certain purple microorganisms bacteria, *Beggiatoa* etc.

The discussion so far focused on classification of bacteria based upon their nutritional requirements. What about fungi? Lets get to know about them.

Fungi are filamentous, eukaryotic microorganisms, ubiquitous in nature. These grow best in dark and moist habitats. Their habitats are diverse. While few are aquatic, most are terrestrial. These are present almost everywhere where the organic material is present and play a very important role in mineralization. Most fungi are saprophytes, i.e., grow on dead organic materials while few are parasitic i.e., grow on plants, animals and humans. Fungi are *chemoorganotrophs*, i.e., use organic compounds as a source of carbon, electrons and energy. These have simple nutritional requirements and can be easily grown in laboratory on simple media. Fungi are found as common contaminants of food, on microbial culture media, paper, cloth and moist surfaces.

Most of the fungi are aerobic (i.e., grow in the presence of oxygen). Some may be obligate anaerobes (i.e. thrive in the absence of oxygen or free air e.g. those found in rumen of cattle). Yeasts are, however, facultative anaerobes (i.e. survive either in the presence or absence of oxygen) and found in habitats rich in sugar. These are unicellular fungi which can obtain energy by fermentation.

Understanding of the nutritional requirement of the organism is therefore needed for its cultivation and maintenance in the laboratory. These nutritional needs can be supplied in the laboratory through culture media.

Now that we know about the nutritional requirements of microorganism, let us next see how these requirements are met by preparing culture media in the laboratory. First we will learn about the types of media and then discuss the composition of some commonly used culture media in microbiology laboratory.

3.4 CULTURE MEDIA AND ITS TYPES

A culture medium (Pl. media), we already know, is a solid or liquid preparation containing all the nutrients required by microorganisms for growth. It is used to grow, transport and store microorganisms. Liquid culture medium is called *broth*. It can be solidified by adding solidifying agent agar-agar in the ratio of 1.5 – 2.0% for complete solid agar and less than 1% for semi-solid medium. What is agar-agar? Agar-agar is a sulphonated mucopolysaccharide containing mainly D-galactose, D-glucuronic acid and 3,6 anhydro L-galactose. It is derived from red sea weed e.g., Gelidium and was introduced to microbiologists by *Fannie Hesse*, wife of *Wather Hesse*. You may recall studying about agar in the Principles of Food Science Course in Unit 2 'Food Polysaccharides and their Applications'.

There are many characteristics, which make agar-agar, an ideal solidifying agent. These are:

(a) It has high gel strength, so it can be used at a concentration that won't inhibit microbial growth due to low water activity.

(b) It solidifies at 40^{o}C and once solidified it can liquefy at 85-90^{o}C. So it is ideal for isolation of thermophiles.

(c) It does not have nutritional value. It is used as a nutrient source only by some marine bacteria, so that in normal use, it is not liquefied by bacterial growth.

Solid media provides a hardened surface on which microorganisms can be grown in the form of discrete colonies to get pure cultures. Solid medium can be used in petri plates as agar plates [refer to Figure 3.1(a)] or in test tubes in form of slants or deep agar tubes, as shown in Figure 3.1(b) and (c).

Figure 3.1: Solid medium

Liquid medium is used in tubes or in flasks.

On the basis of composition, culture media can be categorized into two groups:

(A) *Chemically defined or synthetic media* – Synthetic media are those media where detailed composition of the medium is known in terms of the chemical nature of the ingredient and the quantity present. Preparation of these media needs the knowledge about specific nutritional requirement of the organisms. These media are used widely in research. Example: Inorganic synthetic media, glucose salt broth, BG-11 medium, etc.

(B) *Complex or Undefined Media* – Here, the exact composition of the medium is not known. It contains some complex components of plant and animal extracts whose exact chemical composition is not known. For example, undefined components, like, peptone, beef extract, yeast extract etc. may be present in complex media. These are useful because (i) many microorganisms can grow on the same media, and (ii) it can be used when nutritional requirement of a particular microorganism is unknown. Example: yeast extract broth, nutrient broth, potato dextrose agar etc. Let us get to know a little more about these specific complex media.

(a) *Peptones* – Peptones are protein hydrolysates obtained by partial digestion of meat, casein, soya meal, gelatin or other protein source. These provide carbon, energy and nitrogen.

(b) *Beef Extract and Yeast Extract* – These are aqueous extracts of lean beef and brewer's yeast, respectively. Beef extract provides amino acids, peptides, nucleotides, organic acids, vitamins and minerals. Yeast extract is a source of vitamin B, nitrogen and carbon compounds.

Depending upon the purpose, media can be –

(a) General purpose media, or

(b) Specialized media

Let us get to know about these media.

General purpose media – These support the growth of many microorganisms. Example: nutrient agar, trypic soy agar etc.

Specialized media – These are used for isolation of bacterial types from a mixed microbial population, for differentiation among closely related bacterial groups, enumeration of bacteria, assay of naturally occurring substances etc. These media can be grouped as –

(a) *Selective Media* - It is used to select specific groups of bacteria by favouring the growth of desired bacteria and inhibiting the growth of undesired. Selection can be based on pH, water activity, nutritional content or presence of inhibitors. Example: Crystal violet, bile salts etc. inhibits gram positives while NaN_3, thallous acetate, lithium chloride inhibits gram negatives. Example: oxytetracycline glucose yeast extract agar is selective for yeasts and moulds.

(b) *Enrichment Media* – It contains selective ingredients in the medium, which shifts the growth in a mixed microbial population to a particular group of microorganisms. Example: Selenite broth is an enrichment media for *Salmonella*.

(c) *Differential Media* – It allows differentiation among morphologically and biochemically related group of organisms. It contains certain ingredients which are changed because of microbial metabolism and this change can be seen in form of change in opacity of agar, change in pH or change in colour of media or colonies. Example: Blood agar, mannitol salt agar.

(d) *Selective and Differential Media* – These are used for isolation of specific organisms. These are designed to isolate a group of closely related organisms and differentiation between them. Example: MacConkey agar, eosin methylene blue agar.

(e) *Enriched Media* – It is used for isolation of fastidious organisms, i.e., organisms having specific and elaborate nutritional requirements. Example: Yeast extract broth, Blood agar.

(f) *Living Media* – Some cells will grow only on living cells. So living cell cultures can be used for their cultivation. Example: Viruses.

(g) Various other media like potato dextrose agar, Czapek-dox agar, Sabouraud agar, Rose Bengal agar, antibiotic plate count agar, etc. can be used for cultivating fungi and yeasts. Commonly used medium in the laboratory for growing yeast and fungi is potato dextrose agar. Malt Yeast Peptone Glucose (MYPG) agar is also used for yeast cultivation.

Now that we know about the different media and their types, it is also important to learn about how to make these media. The next section focuses on this.

3.5 COMPOSITION OF SOME COMMONLY USED MEDIA

The composition and procedure for preparation of some commonly used media, which we will use in the laboratory for conducting the exercises is presented in this section. Carefully go through this section and try preparing the media. We begin with nutrient agar and nutrient broth.

1. **Nutrient Agar and Nutrient Broth**

Nutrient Broth

Peptone	-	5.0 gm
Beef Extract	-	3.0 gm
Distilled water	-	1000 ml
pH	-	7.0

For making nutrient agar, add 15 gm of agar into nutrient broth. Nutrient agar and Nutrient broth is a general purpose medium of complex nature. It is widely used in laboratories for cultivation and maintenance of bacteria. It contains peptone and beef extracts, which are the source of various amino acids and vitamins etc. Number of different bacterial types can be easily grown over nutrient medium. It is capable of supporting the growth of most heterotrophs.

2. **Blood Agar**

Infusion from beef heart	-	500 gm
Tryptose	-	10.0 gm
Sodium Chloride	-	5.0 gm
Agar	-	15 gm
Distilled water	-	1000 ml
pH	-	7.3

Dissolve above ingredients and autoclave. Cool to 45-50^{o}C and add 50 ml of sterile defibrinated blood aseptically. Rotate to mix thoroughly and pour immediately into sterile plates or tubes before solidification.

3. **Eosin – Methylene Blue (EMB) Agar (Levine)**

Peptone	-	10.0	gm
Lactose	-	5.0	gm
Dipotassium Hydrogen Phosphate	-	2.0	gm
Agar	-	13.5	gm
Eosin Y	-	0.4	gm
Methylene blue	-	0.065	gm
Distilled water	-	1000	ml
pH	-	7.2	

Dissolve the ingredients and autoclave at 15 psi before dispensing it into sterile plates and tubes. The medium is used for distinguishing lactose fermenting gram negatives from non-lactose fermenting types.

4) **MacConkey Agar**

Bactopeptone	-	17.0	gm
Protease Peptone	-	3.0	gm
Lactose	-	10.0	gm
Bile salt mixture	-	1.5	gm
Sodium chloride	-	5.0	gm
Agar	-	13.5	gm
Neutral Red	-	0.03	gm
Crystal Violet	-	0.001	gm
Distilled water	-	1000	ml
pH	-	7.1	

MacConkey Agar is a specialized media used for selection and differentiation among different bacterial species. It contains crystal violet, which inhibits the growth of gram positive but permits gram negative to grow (selection). Presence of lactose, bile salts and neutral red in medium allows differentiation of bacteria on basis of their ability to ferment lactose. This medium is used to differentiate members of Enterobacteriaceae, which are morphologically and biochemically related into 2 groups.

1) *Lactose Fermenters* – These produce acid on lactose fermentation, which results in red colouration on bacterial colony or surrounding medium. Example *E.coli*.

2) *Non lactose Fermenters* – These do not ferment lactose, so there is no production of acid and colonies appear uncoloured. Example: *Salmonella, Shigella* etc.

5) **Potato Dextrose Agar**

This media is used for cultivation of yeasts, fungi and moulds.

Potato (Peeled)	-	200.0	gm
Dextrose	-	20.0	gm
Agar	-	15.0	gm
Distilled water	-	1000.0	ml
pH	-	5.6	

Peel off the potatoes. After cutting into small pieces, boil them in 500 ml distilled water and filter the extract through cheesecloth. Mix all the ingredients to the filtrate and make the volume to 1000.0 ml by distilled water. Potato dextrose agar is a non-selective and non-differential medium having potato infusion which encourages growth of yeasts and moulds. It contains potato tubers extract, dextrose, peptone and agar. Addition of tartaric acid (1.85 ml of 10% tartaric acid per 100 ml of PDA) lowers its pH and make it inhibitory for the growth of most of the bacteria.

6. **Sabouraud Agar**

Sabouraud agar is used for cultivation of yeasts and moulds.

Peptone	-	10.0	gm
Dextrose	-	40.0	gm
Agar	-	15.0	gm
Distilled water	-	1000	ml
pH	-	5.6	

Dissolve all the ingredients except agar in distilled water. Adjust the pH to 5.6. Add the agar and dissolve by heating. Dispense the media into flasks and autoclave. For preparing Sabouraud agar supplements with aureomycin, aseptically add 10 µg/ml of aureomycin to the sterile molten and cooled media.

7. **Malt Yeast Peptone Glucose (MYPG) Medium**

Malt Extract	-	3.0	gm
Yeast Extract	-	3.0	gm
Peptone	-	5.0	gm
Glucose	-	10.0	gm
Agar	-	15.0	gm
Distilled water	-	1000	ml
pH	-	5.6	

Malt yeast peptone glucose medium is used for cultivation of yeasts and moulds. It is also prepared by dissolving all the ingredients in distilled water and adjusting the pH. Sterilization is done by autoclaving at 15psi before dispensing in sterile plates and tubes.

We have studied about the different media and the ingredients, which go into the making of these media. Surely now you should be able to make these media. Exercises 1-3 in this practical will give you hands on experience of preparing these media and growing the microorganism cultures on these media. But first answer the review questions given herewith.

3.6 REVIEW QUESTIONS

1. What are the nutrient components required by microorganisms?

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2. Name the different nutritional types of microorganisms, giving examples.

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3. What is media? What is the difference between chemically defined and complex media?

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4. Write the purpose and example of following media:

   - Enriched Media .....
   - Selective Media .....
   - Differential Media .....
   - Enrichment Media .....

5. What is Agar? What percentage of agar is used for solid and semi-solid media?

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6. Write the composition of nutrient agar and potato dextrose agar.

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Now, we move on to the Exercise 1.

EXERCISE 1

PREPARATION OF GENERAL PURPOSE MEDIA

Date : .....

Aim: To prepare general purpose media (Nutrient Agar and Nutrient Broth) for cultivation of different bacterial species.

Requirements

Culture : *E. coli, Bacillus, Pseudomonas* and *Staphylococcus.*

Ingredients : Peptone, beef extract, agar, distilled water.

Equipments and Glassware : pH meter, magnetic stirrer, autoclave, hot air oven, bunsen burner, conical flask, measuring cylinder, petri plates and tubes.

Principle:

You have already studied about the principle and ingredients required for preparing nutrient agar in section 3.5. Based on your understanding write the principle and ingredients you would require for preparing the general purpose media in the space provided herewith.

Procedure:

Now prepare the media following the steps enumerated herewith:

1. Weigh and dissolve each ingredient except agar in 500 ml of distilled water.
2. Make the volume to 1000 ml with distilled water. Set the pH to 7.0. This is a nutrient broth.
3. Autoclave the nutrient broth and dispense in sterile test tubes aseptically.
4. For nutrient agar, add 15 gms of agar in 1000 ml of nutrient broth. Heat the mixture to dissolve agar.
5. Dispense the mixture into flasks and autoclave at 15 psi for 15-20 minutes.
6. After autoclaving, cool the nutrient agar medium to 45^{o}C and dispense it aseptically into sterile petri plates and tubes to make agar plates, slants and deep tubes.
7. Allow to solidify. For making slants, keep the tubes in inclined position for agar solidification.
8. Inoculate nutrient agar plates and tubes and nutrient broth with bacterial culture and incubate for 24 hours.
9. Note the observations and results.

Precautions:

1. Dissolve ingredients one by one in distilled water.
2. Check and set the medium to appropriate pH.
3. Autoclave the medium properly.
4. Dispensing of the medium should be done aseptically.

Observations and Results:

Record your observations of the media and the culture formed on the media in the format below:

Observations	Results

Inference/Conclusion:

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Also answer the questions given herewith:

1. Why nutrient agar is called general purpose medium?

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2. What is the composition of nutrient agar?

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3. Why heterotrophs prefer complex media?

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Now submit your exercise for evaluation.

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Counsellor Signature

EXERCISE 2

PREPARATION OF SELECTIVE AND DIFFERENTIAL MEDIUM

Date : .....

Aim : To prepare selective and differential medium-MacConkey Agar – for selection and differentiation of given microorganisms.

Objective : After undertaking this exercise you will be able to

Prepare MacConkey agar medium, which is a selective and differential medium.

Requirements

Culture : 24 hours culture of *Bacillus*, *Pseudomonas*, *Enterobacter*, *E.coli* and *Staphylococcus*.

Chemicals : Bactopeptone, protease peptone, lactose, bile salt, Sodium chloride, Neutral red, Crystal violet, agar.

Equipments and Glassware : Flask, petri plates, autoclave, hot air oven, inoculating loop, B.O.D. incubator.

Theory/Principle

Look up sections 3.4 and 3.5 which presents the composition and method of preparation of MacConkey agar. Based on your understanding write about the media composition, preparation and principle in the space provided herewith.

Selective and Differential Media – Basic Concept

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Composition of MacConkey Agar Medium

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Procedure:

Now prepare the MacConkey agar medium following the steps given herewith:

1. Weigh and dissolve all ingredients except agar one by one in 500 ml of distilled water.
2. Make the volume to 1000 ml with distilled water and set the pH to 7.3.
3. Add agar and dissolve it by heating.
4. Autoclave the medium at 15 psi for 15-20 minutes.
5. After cooling to 45^{o}C, dispense the medium aseptically into sterile petri plates.
6. Allow to solidify and then inoculate with the given cultures.

For inoculation, divide the plate into different sections by marking the bottom of the dish. Label each section with the name of the organism to be inoculated. Inoculate the plate with different organisms by making a single line of inoculation of each organism at appropriate section/place as shown in margin Figure.

7. Incubate the plates in inverted position for 24 hours at 37°C and then record observations.

Precautions:

1. Dissolve ingredients one by one.
2. Check and set the medium to appropriate pH.
3. Autoclave the medium properly.
4. Dispensing of the medium should be done aseptically.

Observations and Results:

Record your observations related to the preparation of media and the culture grown on the media in the format given herewith:

Observations	Results

Inference/Conclusion (Comment on the media and culture formed on the media and its features)

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Answer the questions given herewith.

Review Questions

1. What is the composition of MacConkey media?

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2. For what purpose the MacConkey agar is used?

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3. What is the role of following ingredients in MacConkey agar: Lactose, Crystal Violet, Neutral Red and Bile Salts?

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Submit the exercise for evaluation.

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Counsellor Signature

EXERCISE 3

PREPARATION OF CULTURE MEDIA FOR YEASTS AND MOULDS

Aim : To prepare media for the cultivation of yeasts and moulds. Date : .....

Requirements

Culture : Culture of *Aspergillus, Penicillium, Alternaria* and *Saccharomyces* species.

Ingredients : Malt extract, yeast extract, peptone, glucose, agar, potato tubers, dextrose, distilled water.

Equipments and Glasswares : Erlenmeyer flasks 500 ml, measuring cylinder, beaker, weighing balance, laminar flow chamber, muslin cloth, petri plates, test tubes, petri plate cans, autoclave, incubator, bunsen burner, inoculating needle and loop, cotton (nonabsorbent).

Principle/Theory

Look up section 3.5 which illustrates the composition and preparation of different media. Based on your understanding, write the name of the media used for cultivation of yeasts and moulds and its composition, preparation and principle in the space provided herewith.

Procedure:

Now carry out the exercise following the steps given herewith:

1. To prepare potato dextrose medium, first, peel off and chop 200 gms of potato tubers and then boil in a beaker containing 500 ml of distilled water for 20 minutes.
2. Filter the potato extract with muslin cloth and collect the filtrate.
3. Mix potato extract with other ingredients, i.e., peptone and dextrose and make the volume 1000 ml by distilled water. Adjust the pH by adding 1 N NaOH / 1 N HCl drop-wise.

4. Add 15 gm of agar and dissolve it by heating. Finally, pour it into 3 or 4 Erlenmeyer flasks of 500 ml capacity. Close the mouth of flasks by cotton plugs and then cover it with brown paper or aluminium foil.

5. Sterilize the medium by autoclaving at 15 psi for 15-20 minutes.

6. When the medium cools to 45-50^{o}C, pour it in sterile petri plates for agar plates and in sterile test tubes for slants. Allow it to solidify.

7. For point inoculation, hold the inoculating needle in right hand and sterilize it red hot by keeping it in bunsen burner.

8. Hold the culture tube in left hand and take out the cotton plug with little finger of right hand. Sterilize the mouth of the tube by passing through the flame. Take out the small quantity of the inoculum with sterile inoculating needle after cooling it by touching to the uninoculated medium.

9. Handle the PDA plate in left hand and open the petri plate slightly with the help of thumb. Inoculate it at one point in the centre of the medium. Close the lid and keep the plate for incubation at 28 ± 2^{o}C. Repeat the process for other fungal cultures. Observe after 3-5 days.

Precautions:

1. Plates should be stored at room temperature on bench counter for 24-48 hours to allow free moisture on the agar surface to dry and observe for any contamination.

2. Aseptic conditions should be maintained.

Observations and Results:

Record your observations related to the media prepared and the culture grown on the media in the format given herewith:

Observations		Results
Culture	**Colony appearance**	

Inference/Conclusion:

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Now let us answer the following questions:

Review Questions:

1. What is the nutritional requirement of the fungi?

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2. Which media can be used for fungal and yeast cultivation?

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3. What are the constituents of PDA medium?

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Now submit the exercise for evaluation.

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Counsellor Signature