UNIT - III

M.Sc Applied / M.Sc Microbiology Isem

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Expt. No. 17 Page No. 1

AIM: Preparation of selective and diagnostic media for cultivation isolation, enumeration and purification of micro-organisms.

PRINCIPLE: The term culture is usually employed for deliberate growth of micro-organisms in laboratory. A culture is initiated by placing a small amount of micro-organism as inoculum into sterile media in a flask / test tube or a petriplate. If the liquid is a complete extract of biological material, it is called as broth. It provides nutrients for their growth and to sustain life.

Cultures may vary in form and composition depending upon specific species to be cultured. Some media contain complex ingredients such as extract of plant and animal tissue. Agar is used as a solidifying agent which liquifies on heating to 90°C and hardens into jelly when cooled at 45°C.

Depending upon the composition, there are 3 types of media:

1) Natural Media: These include natural substances such as milk, urine, diluted blood, vegetable juices, meat extract or infusions etc. Among meat extract and infusions, beef extract and infusions are made by infusions

of fresh brown meat in water which are common ingredients and useful for many species.

2. Semi-synthetic media : It is a complex type which contains various ingredients of unknown composition and are useful for cultivation of wide range of micro-organisms. eg- blood, groups of cells as tissue or an organ used for growing viruses, rickettsia etc.

3. Synthetic media : These consists wholly of dilute, reproducible solution of chemicals pure, inorganic compounds. Artificial media of exactly known reproducible composition are called synthetic or chemically defined media.

In modern laboratories, dehydrated medias are used. These are prepared simply by adding weighed composition of the ingredients to the required amount of water.

REQUIREMENTS: Beakers, measuring cylinder, conical flasks, cotton, petriplates.

PROCEDURE :

1. NUTRIENT AGAR MEDIA :-

i) All the components were weighed as per requi-

Teacher's Signature : .....

1. Nutrient Agar Media -

MATERIAL	AMOUNT
Peptone	5g
Beef Extract	3g
NaCl	5g
Agar	15g
Distilled water	1000 ml
PH	7

2. Potato Dextrose Agar -

MATERIAL	AMOUNT
Potato	200g
Dextrose	20g
Agar	15g
Distilled water	1000 ml
PH	5.6

Ref:- Oluf L. Gamborg, Gregory C. Phillips Media preparation & handling, Plant cell, Tissue and organ culture pp 21-34

3. Czapek - Dox Agar Medium

MATERIAL	AMOUNT
$NaNO_3$	2g
K_2HPO_4	1g
$MgSO_4$	0.5g
KCl	0.5g
$FeSO_4$	0.01g
Sucrose	30g
Agar	15g
Distilled water	1000ml
pH	7.3

→ Dissolve all ingredients except phosphate which is dissolved seperately.

Reference :- Jeffery C. Pommerville
Alcamo's Fundamentals of Microbiology
(Tenth edition) Jones & Bartlett.

4. Eosine - Methylene Blue Agar

MATERIAL	AMOUNT
Peptone	10 g
Lactose.	5 g
K_2HPO_4	2 g
Eosine	0.9 g
Methylene Blue	0.065 g
Agar	15 g
Distilled water	1000 ml
pH	7.2

→ used for distinguishing lactose fermenting and non-lactose fermenting bacteria.

Luria - Bertani (LB) Broth

MATERIALS	AMOUNT
Tryptophan	1gm
NaCl	10 gm
Yeast Extract	5 gm
Distilled Water	1000 ml

pH ≈ 7.5

15gm Agar is added to above composition to make LB Broth.

Reference :- Gerard J. Tortora, Berdell R. Funke, Christine L. Case. Pearson - Microbiology An introduction Benjamin Cummins

Enriched Media →
contain nutrients to support growth of a wide variety of organism, commonly used to harvest as many different types of microbes are present in the specimen. Blood agar is which nutritionally rich whole blood supplements the basic nutrients. Chocolate agar is enriched with heat treated blood (70-75°C) which turns brown & gives the medium the color for which it is named

→ selective media
R used for growth of only selected Microorganism. for e.g if a M.O is resistant to certain antibiotic such as ampicillin or tetracycline then dat antibiotic can be added to the medium to prevent other cells which do not possess the resistance, from growing Media lacking AA such as proline in conjuction with E. coli unable to synthesize it were commonly used by penicillin before the emergence of penemics to map bacterial chrom.

— It also used in cell culture for survival & proliferation of cell & certain properties such as Antibiotic resistance or ability to synthesize metabolite. Specific gene grow in selective medium. the gene termed as Marker

Selective growth media for eukaryotic cells commonly contain neomycin to select cells that hv been transfected with plasmid carrying neomycin resistance gene as a Marker.

e.g (1) Eosin methylene blue contain dye that are toxic to gm +ve bacteria.
(2) YM (yeast extract) has low pH, delaying bacterial growth.
(3) Mac conkey agar is - gm -ve bacteria
(4) Hektoen enteric agar = "
(5) Mannitol salt agar = gm +ve
(6) Baird parker agar = gm +ve staphylococci.

Differential Media / indicator
distinguish one morp type from another growing on same medium. uses biochemical characteristic of a u.o growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin methylene blue) added to the medium to visibly indicate the characteristic of a u.o. R used for detection of M.os & by Molecular biologists to detect recombinant strain of bacteria.
e.g
blood agar - contain bovine heart blood that becomes transparent in the trace of hemolytic streptococcus.
- EMB - for lactose fermentation.
- Granada medium - selective - differential for streptococcus agalactiae which grows as red colonies in this medium. (mannitol salt agar)
- X-gal plate - lac operon mutant
- Mac conkey - Lactose fermentor

Anaerobic Media—

Anaerobic bacteria needs special media for growth coz they need low O_2 content, reduced oxidn red^n potential & extra nutrient. Media for anaerobes may bv supplemented ī nutrients like hemin & vit K. such media also hv been to be reduced by physical or chemical means. boiling the medium serve to expel any dissolved O_2. Addn of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic 0.05% cysteine. before use the medium must be boiled in water bath to expel any dissolved O_2 & then sclead ī paraffin

Robertson cooked Meat Medium that is commonly used to grow clostridium spps contain 2-5 cm column of bullock heart meat & 15ml nutrent broth. Thioglycollate broth contain sodium thioglycollate, glucose cystin, yeast extract & caesin hydrolysal.

Methylene blue is an oxidn-red^n potential indicator that is incorporated into medium. under reduced condn, methylene blu is colorless.

Pure cultures

If bacterial species comprises a suitable kind & proportion of mixed population, isolated as pure culture

Strain = derived from a single colony

Strain derived from a single parent cell, termed as clone.

Method of isolating Pure culture

By means of a bacteriological loop (Streak plate Method)

A portion of mixed culture is placed on the surface of an agar medium & streaked across the surface.

When streaking is properly performed, the bacterial cells will be sufficiently far apart in some areas of the plate to ensure that the colony developing from one cell will not merge with that growing from another.

(2) Pour plate - Spread plate technique

In both of these methods the mixed culture is first diluted to provide only a few cells / millilitre before being used to inoculate media.

→ In the pour-plate method the mixed culture is diluted to directly in tubes of liquid agar medium. The medium is maintained in liquid state at temp of 45°C to allow through distribution of inoculum

The inoculated medium is dispersed into petri dishes, allowed solidify & then incubated. A series of agar plate showing ↓↓ no. of colonies resulting frm the dilution procedure in the pour-plate technique. The pour-plate tech has disadvantage some of the organism R trapped beneath the surface of the medium, wen its gels therefore both surface & subsurface colonies develop. Subsurface colonies can transferred to fresh media by first digging them out of the agar ~ sterile instrument.

disadvantage - organism isolated must withstand 45°C temp of liq agar medium, unsuitable for isolating psychrophilic bacteria

In spread plate method :- Mixed culture is not diluted in the culture medium instead it is diluted in series of tubes ~ sterile liq like d.w. A sample is removed frm each ~~plate~~ tube placed onto agar surface of ~~medium~~ plate & spread on surface by means of sterile rod. (Micromanipulator- used in conjuction with a microscope to pick a single bacterial cell frm mixed culture. Micromanipulator permits the operator to control the movement of micro probe (fine needle so that single cell can be isolated)

Maintenance & Preservation of Pure cultures

(1) large No. of strains refued to as stock culture collections. used for research work. These strain R used for screening of new, potentially effective chemotherapeutic agents, tools for vitamins & AA

an agents for production of vaccine antisera, antibiotics agents, enzymes, organic chemical
so it is necessary dat culture must be store & preserved.

Methods of Maintenance & Preservation.

- Strains can be Maintained by periodically preparing a fresh stock culture from the previous stock culture.
- culture Medium storage temp, time interval at which the transfer are made & vary with the species. Many heterotrophs remain viable for sv weeks or months on a medium like nutrient Agar.

Preservation by overlaying cultures ~ Mineral oil

- Many bacteria can be successfully preserved by covering the growth on a agar slant ~ sterile Mineral oil. The oil must cover the slant completely the oil should be about ½ in above the tip of slanted surface.

Advantage → we can remove some of the orth under the oil ~ transfer needle, inoculate a fresh medium & preserve the original culture.

Preservation by Lyophilization (freeze Drying)

Many bact die if culture become dry.
freeze drying preserve many kinds of bacteria
In this process a dense cell suspension is placed in small vials & frozen at -60°C to -78°C

.. The vials are then connected to a high vacuum. The ice present in the frozen suspension under the vacuum i.e evaporates without first going through a liq H2O phase. This results in dehydration of the bacteria with minimum of damage to delicate cell structures.

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Vials R sealed off under vacuum → stored in a refrigerator.

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Many species of bacteria for 30 years

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Minimum storage space is required
(Hundreds of lyophilized cultures can be small area)

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lyophilized cultures are revived by open vials adding liq medium, transfer the rehydrated culture to suitable media.

Reference Book :- Lansing M. Prescott, John P. Harley and Donald A. Klein, Microbiology Mc. Graw Hill companies.