Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY LABORATORY

EXPERIMENT # 2

AIM

To prepare bacterial culture media for growth

BACKGROUND

Introduction

Microrganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes have adapted to the habitats most suitable for their needs, in the laboratory, however, these requirements must be met by a culture medium. This is basically an aqueous solution to which all the necessary nutrients have been added. Depending on the type and combination of nutrients, different categories of media can be made.

Types of Media

1) Complex media / Non-synthetic media

Complex media are rich in nutrients, they contain water soluble extracts of plant or animal tissue (e.g., enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar, often glucose is added to serve as the main carbon and energy source. The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called complex.

2) Defined media / Synthetic media

Defined media are media composed of pure ingredients in carefully measured concentrations dissolved in double distilled water i.e., the exact chemical composition of the medium is known. Typically, they contain a simple sugar as the carbon and energy source, an inorganic nitrogen source, various mineral salts and if necessary growth factors (purified amino acids, vitamins, purines and pyrimidines).

Selective/differential media are media based on either of the two categories above supplemented with growth-promoting or growth-inhibiting additives.

The mixture of necessary nutrients can be used as a liquid medium, or a solidifying agent can be added. "Agar agar" is a natural polysaccharide produced by marine algae and is the most commonly used solidifying agent added to media (end concentration usually 1.5 % w/v). If hydrolysis of the agar is suspected, a silica gel is used as a replacement solidifying agent. The most commonly used media for cultivation of bacteria is nutrient broth/ agar, for cultivation of fungi is potato dextrose agar and for yeast is Malt extract-peptone-dextrose agar.

PREPARATION OF MEDIA

Liquid media/broth: For propagation of large numbers of microbes, fermentation slides

Solid media : For propagation of large numbers of microbes, fermentation studies

Semi-solid media : For motility studies. More jelly like texture, less concentration of agar or gelatin

NUTRIENT BROTH / AGAR

Beef extract	3g
Peptone	5g
NaCl	5g
Distilled water	1L

MGYP BROTH / AGAR

Malt extract	3g
Yeast extract	5g
Glucose	10 g
Peptone	10 g
Distilled water	1L

For BROTH

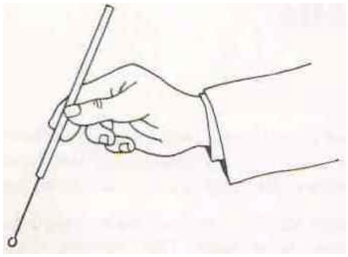
a) Weigh the above ingredients, add it in an Erlenmeyer flask and dissolve in 800 ml of distilled water.
b) Adjust pH 7.2.
c) Make up the volume to 1 L.

For AGAR

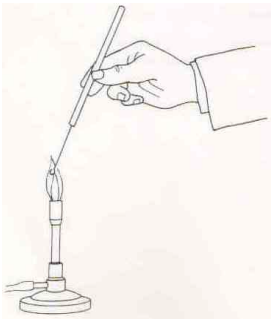
a) To the above preparation add 2% w/v agar-agar.
b) Plug the flask with cotton plug.
c) Sterilize by autoclaving at 15 psi/ 121°C for 15 minutes.

ASEPTIC METHOD FOR TRANSFERRING CELLS

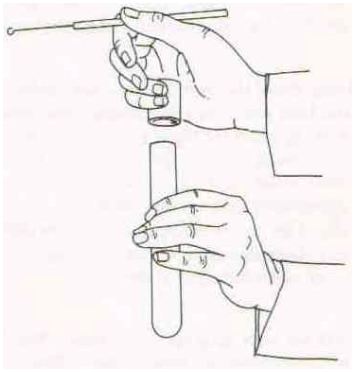
Grasping the inoculation loop



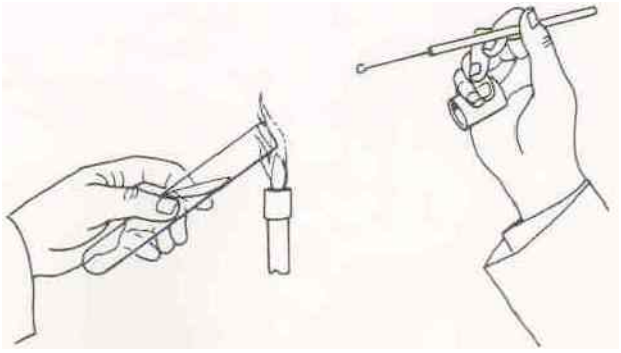
Holding the inoculating loop at a 60 degree angle in the hottest part of a Bunsen burner flame



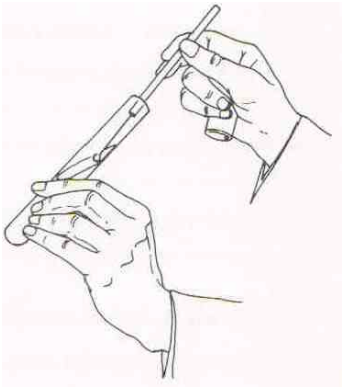
Removing the plug or cap of the tube



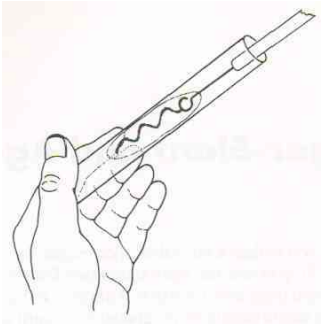
Flaming the tip of the tube



Removing a small amount of culture from the tube



Transfer of culture to Agar slants



Transfer of culture to Streak plates