

MEDIUM FOR ISOLATION OF PLANT PATHOGENIC BACTERIA

Plant pathogenic bacteria, like all other living organisms, require basic nutrients, for the sustenance of life. The food material on which bacteria are grown in the laboratory is known as a culture medium (plural: media) and the growth itself is called a culture. In other words, the nutrient preparation on or in which a culture (i.e., a population of bacteria) is grown in the laboratory is called a culture medium. Although the bacterial pathogens have the same basic requirements, there is a diversity as to the use for organic and inorganic compounds. Thus culture media vary in form and composition, depending on the species to be cultivated. Some media contain solutions of inorganic salts and may be supplemented with the organic compounds while other media contain complex ingredients such as extracts or digests of plant and animal tissues. These gradients, except for the agar, are used to prepare broth or liquid media. Agar, which liquefies on heating to 96°C and hardens into a jelly at 40–45°C, is used to solidify liquid media. Media have been classified variously using different criteria, viz., chemical composition, physical state, and utility purpose. On the basis of their composition, there are three main types of culture media:

1. Natural or empirical culture media
2. Semi-synthetic media
3. Synthetic or chemically defined culture media

The exact chemical composition of a natural medium is not known. Those media whose chemical composition is partially known are called semi-synthetic media. A medium that contains agar becomes a semi-synthetic medium, such as dextrose agar, Czapek–Dox agar, oatmeal agar, and beef peptone agar. Synthetic or a chemical-defined culture medium is composed of special substances of known composition. The synthetic medium may be a general-purpose medium used for a wide variety of microorganisms; a selective medium used for a selected microbe; a differential medium used for differential isolation of a microbe; or an assay medium used for the assay of vitamins, amino acids, and antibiotics. Thus, several media are available and each formulation presumably offers some advantage for the isolation, maintenance, characterization, or growth of certain groups of organisms.

BASIC LIQUID MEDIA (BROTH) FOR THE ROUTINE CULTIVATION OF BACTERIA

Bacteria, in contrast to fungi, are often cultured in a liquid broth (i.e., media lacking agar). The most common constituents of basic media used in a routine bacteriological laboratory are beef extract (a beef derivative which is a source of organic carbon, nitrogen, vitamins, and inorganic

salts) and peptone (a semi-digested protein). These may be modified in a variety of ways by supplementing with some specific chemicals or materials to provide a medium suitable for the cultivation or demonstration of a reaction for specific types or groups of bacteria. Nutrient broth and glucose broth have been considered as basic liquid media for cultivation of bacteria.

Constituents for Nutrient Broth (pH 7.0)

Peptone 5.0 g

Beef extract 3.0 g

Distilled water 1000.0 ml

Constituents for Glucose Broth (pH 7.3)

Peptone 10.0 g

Glucose 5.0 g

Sodium chloride 5.0 g

Distilled water 1000.0 ml

Material Required

Ingredient of the respective medium, 1 N HCl, 1 N NaOH, pH meter, distilled water, hot plate/heater, autoclave, beaker (1 L), measuring cylinder, cotton, culture tubes, glass rod.

Procedure Preparation of Nutrient Broth

Dissolve the given quantity of peptone and beef extract in 1 L of distilled water for preparation of nutrient broth and boil on the gas burner till all the ingredients are dissolved and mixed well. Adjust the pH to 7.0 and sterilize in an autoclave at 15 pounds of pressure for 30 minutes.

Preparation of Glucose Broth Dissolve the given quantity of peptone, glucose, and sodium chloride in 1 L of distilled water for preparation of glucose broth and boil on the gas burner till all the ingredients are dissolved and mixed well. Adjust the pH to 7.3 and sterilize in autoclave at 15 pounds' pressure for 30 minutes.