

PREPARATION OF MEDIA AND CULTURE FOR PATHOGENIC BACTERIA

Materials Required:

- Dehydrated nutrient agar
- Dehydrated nutrient broth
- A balance, and weighing papers
- Conical flask, cotton plugged or screw capped
- beaker
- Measuring cylinder
- Pipettes
- Test tubes (screw capped or cotton plugged)
- Petri dishes
- Laminar air flow
- Autoclave

Media preparation and sterilization

All the glassware used for the bacteriological works were sterilized in hot air oven at 160°C for 2 hrs. Media, tips for micropipette, micro centrifuge tubes, saline etc. were sterilized using autoclave at 121°C under 15 lb pressure for 15 min. Media containing glucose and some specific heat sensitive constituents (which cannot be sterilized at 121°C) were sterilized by autoclaving at 10 lb pressure for 20 min.

Sample preparation

10 gm meat from the pooled sample was aseptically transferred to sterile pouches and homogenised with 90 ml enrichment broth. 150 ml each water samples were collected using sampling bottle and brought at chilled condition and out of that 10 ml was used for enrichment (directly added into 90 ml enrichment broth).

Enumeration of Total Plate count (TPC)

For enumerating Total Plate count (TPC)- 10 gm each pooled samples of fish were macerated in stomacher and for that of water sample 10 ml were brought into suspension with 9 volumes of sterile physiological saline (0.85% NaCl). Ten fold serial dilutions of the samples were made and 0.1 ml from each dilution was placed on duplicate plates of nutrient agar plates following spread plate technique followed by the incubation of the plates at 37°C for 24 hrs.

Enrichment and selective plating

Enriched media contain the nutrients required to support the growth of a wide variety of organisms, including some of the more fastidious ones. They are commonly used to harvest as many different types of microbes as are present in the specimen. Enrichment medium is general purpose enrichment agar which can nourish and support the growth of gram-positive as well as gram-negative bacteria. It can also be supplemented with blood for enriched growth or study the haemolysis. This medium contains peptic digest of animal tissue and yeast extract which serves as source of nitrogen, carbon, amino acids, vitamins and growth factors for growth of bacteria. Dipotassium phosphate buffers the medium well. Agar is solidifying agent.

Selective plating

Selective media allow certain types of organisms to grow, and inhibit the growth of other organisms. The selectivity is accomplished in several ways. For example, organisms that can utilize a given sugar are easily screened by making that sugar the only carbon source in the medium. On the other hand, selective inhibition of some types of microorganisms can be achieved by adding dyes, antibiotics, salts or specific inhibitors which affect the metabolism or enzyme systems of the organisms.

For example, media containing potassium tellurite, sodium azide or thallium acetate (at concentrations of 0.1 - 0.5 g/l) will inhibit the growth of Gram-negative bacteria. Media supplemented with penicillin (5-50 units/ml) or crystal violet (2 mg/l) will inhibit the growth of Gram-positive bacteria. Tellurite agar, therefore, is used to select for Gram-positive organisms, and nutrient agar supplemented with penicillin can be used to select for Gramnegative organisms.

Incubation

The plates were incubated at 37°C in an incubation chamber for 24 to 48 hrs. and growth of the colonies was observed. However, sometimes it required 72 hrs as well due to its slow growing nature.

Question: Write the steps involve in the preparation for Total Plate count using Nutrient Agar.