Technical Information

Tryptone Bile Agar

Product Code: DM 1961

Application: - Tryptone Bile Agar is used for rapid detection and enumeration of *Escherichia coli* in foods using a modified direct plating method.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Bile salts mixture	1.500
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Anderson and Baird-Parker [1] formulated Tryptone Bile Agar. The International Commission on the Microbiological Specifications for Foods (CMSF) [2] compared the Most Probable Number (MPN) and the Anderson-Baird-Parker Direct Plating Method (DPM) and observed that DPM was superior to MPN for enumeration of *Escherichia coli* from raw meats. Superiority of DPM method was observed by the organization on the basis of less variability, better recovery from frozen samples, greater rapidity and the smaller quantity of medium required. The DPM enumerates both anaerogenic and late lactose fermenting strains of *E. coli* which could be missed by the MPN method (about 10%) [3]. This formulation is recommended by ISO committee for the enumeration of *E. coli* [4]. Holbrook et al [5] modified the DPM for detection and enumeration of sublethally damaged cells of *E. coli* in frozen, dried, heat processed or acid foods and found that resuscitation step reduces the high concentration of sugar present in the inoculum to a level which does not interfere with the production of indole as the synthesis of tryptophanase is inhibited by high sugar concentrations [6].

Certain organism's breakdown the amino acid tryptophan with the help of enzymes that mediate the production of indole by hydrolytic activity [7]. The indole produced can be detected by either Kovacs or Ehrlichs reagent [8]. Indole combines with the aldehyde present in the above reagent to give red colour in the alcohol layer. The alcohol layer extracts and concentrates the red colour complex. The indole positive organisms other than *E. coli* are inhibited by bile salts and higher incubation temperature.

Methodology

Suspend 36.5 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 3.65% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH range

7.00-7.40

Cultural Response/Characteristics

DM 1961: Cultural characteristics observed after an incubation at 44°C for 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Enterobacter aerogenes ATCC 13048	>=10^3	inhibited	0%
Escherichia coli ATCC 25922	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Anderson J. M. and Baird-Parker A. C., 1975, J. Appl. Bacteriol., 39:111.
2. International Commission on Microbiological Specifications for Food, 1979, Can. J. Microbiol., 25:1321.
3. Ewing W. H., 1972, US Dept. of Health, Education and Welfare, CRC, Atlanta.
4. International Organization for Standardization (ISO), 1988, Draft ISO/DIS 6391.
5. Holbrook R., Anderson J. M. and Baird - Parker A.C., 1980, Food Technol. in Aust., 32:78.
6. Clarke P. H. and Cowen S. T., 1952, J. Gen. Microbiol., 6:187.
7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

Finegold S. M., Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

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