Technical Information

Dihydrolase MiVeg Broth Base

Product Code :VM1915

Application:- Dihydrolase MiVeg Broth Base is used to study the dihydrolase reaction of *Vibrio parahaemolyticus*.

Composition

Ingredients	Gms / Litre
MiVeg peptone	5.0
Yeast extract	6.0
Dextrose	2.0
Sodium chloride	30.0
Bromo cresol purple	0.032
Final pH (at 25°C)	6.8±0.2

** Formula adjusted, standardized to suit performance parameters.

Principle & Interpretation

Dihydrolase MiVeg Broth Base is prepared by using MiVeg peptone instead of Peptic digest of animal tissue used in the conventional medium which makes it free from BSE/TSE risk[2]. This media is the modification of Dihydrolase Broth Base which is formulated as per APHA (1) and is used for studying dihydrolase reaction of *Vibrio parahaemolyticus* from other *Vibrio* species. It is supplemented with L-Arginine as a substrate for dihydrolase reaction (2, 3).

Dihydrolase enzyme converts L-Arginine to putrescine however putrescine is also formed from arginine by the decarboxylase system. In the decarboxylase system, L-Arginine undergoes decarboxylation to yield agmatine. Then this Agmatine is catabolized by the enzyme agmatine dehydrolase to putrescine, Co_2 (Carbon dioxide) and ammonia by way of an intermediate compound monocarbaminyl putrescine (4). This process occur in two steps. In the first step, hydrolytic removal of NH_2 from arginine takes place by the action of an arginine dihydrolase and arginine desimidase to yield citrulline, ammonia and inorganic phosphate (5). In the second step citrulline undergoes splitting or phosphorelytic cleavage by citrulline ureidase to yield ornithine and carbamylphosphate. Then further Ornithine is decarboxylated to putrescine and carbon dioxide. Thereby the pH is elevated because of production of amine like putrescine in the medium (6). Bromo cresol purple serve as a pH indicator in the medium which turns purple from yellow at alkaline pH. For the confirmation, it is recommended to inoculate into a basal medium tubes which does not contain L-Arginine. Exposure to air may cause alkalinization of the surface of the medium so a dihydrolase negative organism may be misidentified as positive. So it is suggested to protect the inoculated tubes from air by overlaying with sterile mineral oil. This medium contains MiVeg peptone and yeast extract which supplies nitrogenous nutrients to support bacterial growth. Dextrose serve as a fermentable carbohydrate.

Methodology

Suspend 43 grams of powder media in 1000 ml distilled water. Mix thoroughly. Heat if necessary to dissolve the medium completely. Divide in 2 parts. Add 0.5% L-Arginine to first portion. Use second portion as control. Dissolve completely and dispense 3.0 ml into 13 mm x 100 mm screw cap tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance

Light yellow coloured, may have slightly greenish tinge, homogeneous, free flowing powder.

Colour and Clarity of prepared medium

Purple coloured, clear solution without any precipitate.

Reaction

Reaction of 4.3% w/v aqueous solution pH: 6.8±0.2 at 25°C

pH range

6.6-7.0

Cultural Response/Characteristics

Cultural characteristics observed after an incubation at 35-37°C for 18-24hours with added 0.5% L-Arginine and overlaying with sterile mineral oil after inoculation.

Organisms (ATCC)	Inoculum (CFU)	Arginine dihydrolase
Enterobacter aerogenes (13048)	luxuriant	-
Vibrio cholera (15748)	luxuriant	-
Vibrio parahaemolyticus (17802)	luxuriant	+

Key : + = purple to yellow to purple
- = purple to yellow

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. Speck M.L. (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.
2. Moeller V., 1955, Acta Pathol. Microbiol. Scand., 36:158.
3. Slade H.D. and Slamp W.C.,1952, J. Bact., 64:455.
4. Oginsky E.L. and Gehrig R.F., 1953, J. Biol. Chem., 204:721.
5. Sokatch J.R., 1969, Bacterial Physiology and Metabolism, New York : Academic Press, pp. 169.
6. MacFaddin J.F., 2000(ed), Biochemical Tests for Identification of Medical Bacteria, 3rd edition, Lippincott Williams and Wilkins, New York.

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