Crystal Violet Tetrazolium Agar Base

M586

Crystal Violet Tetrazolium Agar is used for detection of gram-negative psychrotrophic bacteria causing food spoilage.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	2.500
Dextrose	1.000
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 5 ml of sterile 1% solution of 2, 3, 5-Triphenyl Tetrazolium Chloride (FD057). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Microorganisms which are able to grow at refrigeration temperatures are usually referred to as psychrophilic. Species of *Achromobacter, Alcaligens, Flavobacterium* and *Pseudomonas* are included among the psychrotrophic bacteria as these organisms are able to grow relatively rapidly at commercial refrigeration temperatures (1). Many psychrotrophic microorganisms when present in large numbers can cause a variety of off-flavors as well as physical defects in foods. Their growth rate is highly dependent on temperature, and therefore, if the temperature is reduced, their growth rate is also slowed down. Thus the spoilage of refrigerated food is very much dependent on temperature (2, 3).

Crystal Violet Tetrazolium Agar Base is used for the detection of gram-negative psychrophilic bacteria causing food spoilage. It is based on the formulation by Olson (4) and recommended by APHA (5) for detecting gram-negative psychrotrophic bacteria.

Casein enzyme hydrolysate and yeast extract provide various nitrogenous nutrients to the organisms while dextrose serves as the carbon source. Crystal violet inhibits most of the gram-positive organisms and therefore inclusion of crystal violet in the medium does not affect the growth of psychrotrophic organisms, which are mostly gram-negative.

Standard methods for the detection of gram-negative psychrotrophic bacteria should be followed (5).

Quality Control

Appearance
Cream to greyish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.35% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M586: Cultural characteristics observed with added 1% T.T.C. solution (FD057) after an incubation at 20-30°C for 18-48 hours

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony

Cultural Response

Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	>=50%	maroon
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	
Yersinia enterocolitica ATCC 27729	50-100	good-luxuriant	>=50%	maroon

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on label.

Reference

1. Mossel D. A. A., and Zwart H., 1960, J. Appl. Bacteriol., 23:185-188.
2. Elliott R. P. and Michener H. D., 1965, U.S. Dept. Agr. Tech. Bull.No. 1320, p. 110, Washington, D.C.
3. Tomkin R. B., 1973, Food Technol., 27:54.
4. Olson H. C., 1963, J. Dairy Sci., 46:362.
5. Speck M. L., (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed., APHA, Washington, D.C.

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