Calcium Caseinate Agar

M1309

Calcium Caseinate Agar is used for the detection and enumeration of proteolytic microorganisms in foodstuffs and other materials.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	4.000
Meat extract	2.000
Casein enzymic hydrolysate	2.000
Calcium caseinate	3.500
Calcium chloride.2H2O	0.200
Tri-potassium citrate, H20	0.350
Disodium hydrogen phosphate	0.105
Potassium dihydrogen phosphate	0.035
Sodium chloride	5.000
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.19 grams in 1000 ml distilled water. Heat gently while frequently shaking until the suspension boils. Boil for 10 minutes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix thoroughly while pouring into sterile Petri plates to suspend the precipitate. If desired, to increase turbidity, add 5-10 grams of skim milk powder before heating.

Principle And Interpretation

Protein hydrolysis by microorganisms in foods may produce a variety of odour and flavour defects. On the other hand, microbial proteolytic activity may be desirable in certain foods such as in the ripening of cheese, where it contributes to the development of flavour, body and texture. In some foods the level of proteolytic microorganisms may be of value to predict refrigerated storage life and to assess processing methods (1, 2). Calcium Caseinate Agar is a modification of the original formulation of Frazier and Rupp (3) and is used for the detection and enumeration of proteolytic microorganisms in foodstuffs and other materials. Casein enzymic hydrolysate provides nitrogenous, carbonaceous nutrients along with vitamins and amino acids. Phosphates are added to buffer the medium. Sodium chloride maintains the osmotic equilibrium. Casein in the medium is degraded by the proteolytic organisms. This results in formation of clear zones around the proteolytic colonies, in the otherwise opaque medium.

The test sample can be directly surface inoculated or the inoculation can be carried out by the pour plate technique. After an incubation for 24-48 hours, proteolytic organisms, if present will form clear zones on the medium. For better visualization of the zones, the plates can be flooded with 5-10% acetic acid.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium
Whitish coloured, turbid gel forms in Petri plates

Reaction
Reaction of 3.02 % w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response

M1309: Cultural characteristics observed after an incubation at 35-37°C for 2 4 -48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Proteolytic activity
Bacillus cereus ATCC 14579	50-100	good-luxuriant	>=70%	positive, clear zone surrounding colonies
Escherichia coli ATCC 25922	50-100	good-luxuriant	>=70%	negative, no clear zone surrounding colonies
Proteus vulgaris ATCC 13315		good-luxuriant	>=70%	negative, no clear zone surrounding colonies
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	>=70%	positive, clear zone surrounding colonies

Storage and Shelf Life

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

Reference

1. Chai T., Chen C., Rosen A. and Levin R. E., 1968, Appl. Microbiol.,16: 1738.
2. Martely F. G., Jayashankar S. R. and Lawrence, R. C., 1970, J. Appl. Bacteriol., 33: 363.
3. Frazier W. C. and Rupp P., 1928, J. Bacteriol., 16, 57-63

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