Sulphite Agar

M311

Sulphite Agar is used for detection of thermophilic sulphide producing anaerobic microorganisms.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Sodium sulphite	1.000
Agar	20.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in screw-capped tubes containing a clean iron nail in 15 ml amounts and cap the tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. As an alternate to iron nail, 10 ml of 5% ferric citrate solution may be used per litre of the medium.

Principle And Interpretation

Oxidation of sulphur or sulphides for energy production is restricted to the bacterial genus *Thiobacillus* , *Thiomicrospira* , and *Sulfolobus* . These bacteria all produce sulphuric acid as a metabolic product. The direct reduction of sulphate ions to hydrogen sulphide is effected in nature by specialized, strictly anaerobic bacteria of the genera *Desulfovibrio* and *Desulfotomaculum* . These sulphate-reducing bacteria (SRB) are heterotrophic organisms that utilize sulphate, thiosulphate, S2O3--, sulphite, SO3--, or other reducible sulphur-containing ions as terminal electron acceptors in their respiratory metabolism. In the process these sulphur-containing ions are reduced to hydrogen sulphide. Sulphite Agar is prepared according to the formula described by Clark and Tanner (1) and is recommended by APHA (2, 3) for detecting the thermophilic hydrogen sulphide producing anaerobic microorganisms.

Casein enzymic hydrolysate in the medium provides nitrogenous compounds required for the growth of organisms. Sodium sulphite is reduced and thus contribute in H2S production by the thermophilic anaerobic bacteria.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Light amber coloured clear to very slightly opalescent gel forms in tubes

Reaction
Reaction of 3.1% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH
7.40-7.80

Cultural Response
M311: Cultural characteristics observed after an incubation at 55°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Sulfite reduction
Cultural Response			
Cl. thermosaccharolyticum ATCC 7956	50-100	good	positive reaction, blackening of medium

Desulfotomaculum nigrificans ATCC 19858	50-100	good	positive reaction, blackening of medium
Bacillus stearothermophilus ATCC 10149	50-100	good	negative reaction, no blackening of medium

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 the label.

Reference

1. Clark F. M. and Tanner F. W., 1937, Food Research, 2:27.
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
3. Horwitz W., (Ed.), 2000, Official Methods of Analysis of AOAC International, 17th Ed., AOAC International, Gaithersburg, Md.

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