Reddys Differential Agar, Modified (Lactic Streak Agar) M926

Reddys Differential Agar, Modified (Lactic Streak Agar) is recommended for qualitative and quantitative differentiation of lactic Streptococci.

Composition**

Ingredients	**Gms / Litre**
Peptic digest of animal tissue	5.000
Papaic digest of soyabean meal	5.000
Yeast extract	5.000
Beef extract	5.000
Lactose	1.500
L-Arginine hydrochloride	1.500
Bromo cresol purple	0.002
Sodium carboxymethyl cellulose	10.000
Calcium citrate	10.000
Agar	15.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58 grams in 1000 ml distilled water and disperse using blender. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 115°C for 10 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The most common microorganisms used in the dairy industry as starter cultures are lactic streptococci. *Lactococcus lactis* and its subspecies *cremoris* and *diacetylactis* belong to this group. These can be differentiated by biochemical tests, the major criteria being arginine hydrolysis and test for diacetyl and acetoin. Reddys Differential Agar, Modified (Lactic Streak Agar) recommended for the qualitative and quantitative differentiation of lactic streptococci was originally described by Reddy et al (1) and further modified by Mullan and Walker (2). This medium is recommended by APHA (3) for the differential enumeration of lactic streptococci.

Lactose fermenters produce acid and form yellow colonies. *Lactococcus lactis* initially produces acid but later on turns to violet-purple colour due to the release of ammonia from arginine. *Lactococcus lactis* subspecies *diacetylactis* produces a more intense purple colour than *Lactococcus lactis* . The former utilizes the suspended calcium citrate and the citrate degrading colonies exhibit clear zones against a turbid background.

Peptic digest of animal tissue, papaic digest of soyabean meal, yeast extract and beef extract serve as sources of essential nutrients including carbon, nitrogen, amino acids and vitamins. Lactose is the fermentable carbohydrate. L-arginine and calcium citrate are the specific substrate. Bromocresol purple is the pH indicator.

For quantitative determination, decimal dilution of cultures are prepared and spread on agar plates. After incubation at 32°C for 36 to 40 hours, yellow colonies of subspecies *cremoris* are counted. The plates are further incubated for 4 days and then total count of colonies with clear zones belonging to subspecies *diacetylactis* are obtained and subtracted from total count to get *Lactococcus lactis* population in the mixture.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured opalescent with greenish tinge forms in Petri plates

Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH
5.80-6.20

Cultural Response
M926: Cultural characteristics observed after an incubation at 32°C for upto 4 days .

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Citrate Utilization
Lactobacillus lactis ATCC 8000	50-100	good-luxuriant	>=50%	yellow	negative reaction
L. lactis subsp.cremoris ATCC 19527	50-100	good-luxuriant	>=50%	purple	negative reaction
Lactococcus lactis subsp.diacetylactis	50-100	good-luxuriant	>=50%	purple	positive reaction, clearing around the colony

Storage and Shelf Life

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

Reference

1. Reddy M. S., Vedamuthu E. R., Washam C. J. and Reinbold G. W., 1972,Appl. Microbiol., 24: 947.
2. Mullan W. M. A. and Walker A. L., 1979, An agar Medium and a simple streaking technique for the differentiation of the lactic streptococci, Dairy Industries. International, 44 (6):13, 17.
3. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed, APHA, Washington, D.C.

Revision : 2 / 2015

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