Reddy's Differential HiVeg™ Agar, Modified (Lactic Streak HiVeg™ Agar)

MV926

Intended Use:

Recommended for qualitative and quantitative differentiation of lactic Streptococci.

Composition**

Ingredients	g / L
HiVeg™ peptone	5.000
Soya peptone	5.000
Yeast extract	5.000
HiVeg™ 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 58.0 grams in 1000 ml purified/distilled water and disperse using blender. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at Δ 115°C for 10 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Δ corresponds to 10 lbs pressure.

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. Reddy's 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. Lactic Streak HiVeg™ Agar is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. HiVeg™ peptone, Soya peptone, yeast extract and HiVeg™ 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.

Type of specimen

Food samples

Specimen Collection and Handling:

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.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Well isolated colonies must be used.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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 between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

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. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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