Lactobacillus MRS Agar (MRS Agar), Granulated

GM641I

Lactobacillus MRS Agar is recommended for the isolation and enumeration of lactic acid bacteria from meat and meat products. The composition and performance criteria of this medium are as per the specifications laid down in ISO 13721:1995 (E).

Composition**

ISO 13721:1995(E) specification

Ingredients	Gms / Litre
Meat extract	8.000
Peptone	10.000
Yeast extract	5.000
Triammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate, heptahydrate	0.200
Manganese sulphate, tetrahydrate	0.050
Dipotassium hydrogen phosphate	2.000
Glucose, anhydrous	20.000
Sorbitan monooleate (Tween 80)	1.000
Agar	12.0-18.0
pH after sterilization (at 25°C)	5.7

Lactobacillus MRS Agar, Granulated **GM641I**

Ingredients	Gms / Litre
HM Peptone B#	8.000
Peptone	10.000
Yeast extract	5.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate, heptahydrate	0.200
Manganese sulphate, tetrahydrate	0.050
Dipotassium phosphate	2.000
Dextrose (Glucose)	20.000
Polysorbate 80 (Tween 80)	1.000
Agar	12.000
Final pH (at 25°C)	5.7±0.2

**Formula adjusted, standardized to suit performance parameters # - Equivalent to Beef extract

Directions

Suspend 65.13 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacilli MRS medium is based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all *Lactobacilli* (1), dairy products (2), foods (3) and other sources (4). Lactobacillus MRS Agar is recommended by ISO Committee (5).

Peptone and HM Peptone B supplies nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Yeast extract provides vitamin B complex and glucose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of *Lactobacilli*. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms. Phosphates provide good buffering action in the media.

Lactobacilli are microaerophillic and generally require layer plates for aerobic cultivation on solid media. When the medium is set, another layer of un-inoculated MRS Agar is poured over the surface to produce a layer plate (4). *Lactobacilli* isolated on MRS Agar should be further confirmed biochemically.

Type of specimen

Water samples; Food and dairy samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

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.Further biochemical tests are required for complete identification.
2.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

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 light yellow coloured granular medium

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Medium to dark amber coloured slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.51% w/v aqueous solution at 25°C. pH : 5.7±0.2

pH

5.50-5.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (longer if neccesary) (with 5% CO_2)

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus acidophilus ATCC 4356	50-100	luxuriant	>=50%
Lactobacillus casei ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	luxuriant	>=50%
Lactococcus lactis subsp. lactis ATCC 19435	50-100	luxuriant	>=50%
Lactococcus sakei ATCC 15521	50-100	luxuriant	>=50%
Pediococcus damnosus ATCC 29358	50-100	luxuriant	>=50%
Pediococcus pentosaceus ATCC 33316	50-100	luxuriant	>=50%
*Bifidobacterium bifidum** ATCC 11863	50-100	luxuriant	>=50%
Escherichia coli ATCC 25922	50-100	none-poor	<=10%

Key : (*) -Growth under anaerobic conditions for 72 hours

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°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 (10,11).

Reference

1.deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.
2.Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington,D.C.
3.Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
4.MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.
5.International Organization for Standardization (ISO), ISO 13721:1995, Meat and meat products- Enumeration of lactic acid bacteria- Colony-count technique at 30°C.
6.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
7.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
9.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
10.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
11.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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