MRS Agar

Cat. 1043

For the growth of lactobacilli

Practical information

Aplications	Categories
Selective enrichment	Lactobacilli

Industry: Food / Alcoholic beverages / Dairy products

Principles and uses

MRS Agar is a selective medium, based on the formulation developed by de Man, Rogosa and Sharpe to provide a medium that would support the good growth of lactobacilli in general, but in particular of those strains which showed poor growth in existing media such as L. brevis and L. fermenti, replacing a variable product (tomato juice).

The medium is apt for the growth of lactic acid bacteria, including Lactobacillus, Pediococcus and Leuconostoc.

Ammonium citrate, at a low pH, inhibits most microorganisms, but allows the growth of Lactobacilli. Dipotassium phosphate and Sodium acetate are buffer agents to maintain a low pH. Tween 80 is an emulsifier. Manganese and Magnesium sulfates are sources of ions and sulfate. Bacteriological peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly the B-group. Dextrose is the fermentable carbohydrate. Bacteriological agar is the solidifying agent.

Lactobacilli are microaerophilic and generally require layered plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery, and are small, opaque and white

Formula in g/L

Bacteriological agar	10	Bacteriological peptone	10
Dextrose	20	Dipotassium phosphate	2
Magnesium sulfate	0,2	Manganase sulfate	0,05
Beef extract	8	Sodium acetate	5
Tween 80	1	Yeast extract	4
Ammonium citrate	2		

Preparation

Suspend 62 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 12 minutes. Cool to 45-50ºC, mix well and dispense into plates.

Instructions for use

For the enumeration of mesophilic acid lactic bacteria:
- Pour 1 ml of the previously diluted sample into a sterile Petri dish.
- Add a first layer of the cooled medium (45-50 °C).
- After solidification, a second layer is poured.
- Incubate the plates up to 3 days at 35°C or up to 5 days at 30 °C. If possible, incubate the plates in a CO_2 atmosphere.
- It is important to maintain a humid atmosphere because the plates should not dry out during incubation.
- The growth of some Lactobacillus strains are inhibited at a higher pH of 6.0 and it is necessary to acidify the media to promote growth. To acidify the media some drops of acetic acid can be added.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	6,2 ± 0,2

Microbiological test

Incubations conditions: (30±1 ºC / 72±3 h).
Inoculation conditions: Productivity quantitative (100±20. Min.50 CFU)/ Selectivity (10^4-10^6 CFU).
Reference media: Media batch MRS already validated.

Microorganisms	Specification
Lactobacillus sakei ATCC 15521	Good growth, >70%
Lactococcus lactis ssp. lactis ATCC 19435	Good growth, >70%
Escherichia coli ATCC 25922	Moderate growth

Storage

Temp. Min.:2 ºC
Temp. Max.:8 ºC

Bibliography

Briggs M (1.953) "An Improved Medium for Lactobacilli" J. Dairy Res. 20. 36-40.
De Man, J.C. Rogosa, M., Sharpe, Elisabeth (1960) "A Medium for the Cultivation of Lactobacilli". J. Appl. Bact. 23. 130-135