Specification

Solid medium for the culture of lactic acid bacteria according to de Man, Rogosa and Sharpe, modified according to ISO standards and IFU methods.

Formula * in g/L

Enzymatic digest of casein	10.00
Meat extract	10.00
Yeast extract	4.00
D(+)-Glucose	20.00
Sodium acetate	5.00
Triammonium citrate	2.00
Magnesium sulphate heptahydrate	0.20
Manganese sulphate tetrahydrate	0.05
Dipotassium phosphate	2.00
Polysorbate 80	1.08
Agar	14.00

Final pH 5.7 ±0.1 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 68.33 g of powder in one litre of distilled water, and bring to a boil. Distribute in suitable containers and sterilized in the autoclave for 15 minutes at 121 ° C.
Avoid overheating that darken the medium and affect the firmness of the gel.
If different of the formulated final pH values are desired, it is recommended to add small amounts of a 1M solution of acetic acid or NaOH, as required, but it should be noted that the selectivity will be altered depending on the pH.

Description

The MRS medium is an improved modification that replaces the media previously used for the cultivation of lactobacilli, all based on the nutritional properties of tomato juice. The addition of magnesium, manganese acetate, and polysorbate enhance the growth of lactobacilli, even the most fastidious species such as *Lactobacillus brevis* and *Lactobacillus fermenti.*
The high quality of peptones and supplements as meat extracts and yeast, provide growth factors necessary to make the MRS one of the most complete for the cultivation of lactobacilli media. But selectivity is scarce and often tends to allow contaminants, which require a greater selection. For this, alternate subcultures on double layer solid medium and broth are recommended. In many cases growth is favored by an atmosphere enriched in CO_2.
MRS medium is especially suited for enumeration and maintenance of lactobacilli in plate by mass inoculation and covering with a second layer of molten medium, which usually avoids the need for the CO_2 enriched atmosphere, especially in the first isolation.
In industrial production of fruit juices and concentrates the medium is used with the final pH adjusted to 5.0 for the control of lactic acid bacteria which can alter the product.
With the pH adjusted to 6.2 ±0.2 and the addition of clindamycin 0.1 mg/l and ciprofloxacin 10 mg/l the medium is used for selective enumeration of *Lactobacillus acidophilus* in dairy products. Without the additives but with the pH adjusted to 5.4 it can be used for enumeration of *Lactobacillus bulgaricus* in yoghurt.

NOTE: After a long static storage the product tends to cake and compact without affecting quality. Its fluidity can be recovered by a strong and vigorous shaking of the capped container.

Technique

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.
Spread the plate by streaking methodology or by spiral method. Incubate the plates right side up in a CO_2 atmosphere at 30 ±1ºC for 72 ±3h.
(Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample , on the specifications,...
This medium can be inoculated directly or after enrichment broth like MRS broth) Incubated under microaerophilic conditions to promote lactobacilli enrichment.
After incubation, enumerate all the colonies that have appeared onto the surface of the agar.
Each laboratory must evaluate the results according to their specifications.
Calculate total microbial count per ml of sample by multiplying the average number of colonies per plate by inverse dilution factor if streaked a diluted sample. Report results as Colony Forming Unit (CFU's) per ml or g along with incubation time and temperature.

Quality control

Incubation temperature: 30 ± 1 ºC **Incubation time:** 72 ± 3 h

Inoculum: Practical range 100±20 CFU. min. 50 CFU (productivity)/ 10^4 CFU (selectivity), according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Lactobacillus sakei ATCC® 15521	Productivity > 0.70	Incubated in a 5% CO_2 atmosphere
Lactococcus lactis ATCC® 19435	Productivity > 0.70	Incubated in a 5% CO_2 atmosphere
Pediococcus pentosaceus ATCC® 33316	Productivity > 0.70	Incubated in a 5% CO_2 atmosphere
Escherichia coli ATCC® 8739	Inhibited	-
Escherichia coli ATCC® 25922	Inhibited	-

References

· DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods.4th Ed. APHA. Washington DC. USA
· FIL-IDF Standard 146 (2003) Yoghurt. Identification of characteristic micro-organisms.
· FIL-IDF Standard192 (2006) Enumeration of presumptive Lactobacillus acidophilus on a selective medium. - Colony-count technique at 37ºC.
· IFU Method No 5 (1996) Lactic Acid Bacteria Count Procedure. Schweizerischer Obstverband. CH-6302 Zug
· IFU Method No 9 (1998) Microbiological examination of potential spoilage micro-organisms of tomato products. Schweizerischer Obstverband. CH-6302 Zug
· ISO Standard 11133 (2014) Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· ISO Standard 15214 (1998) Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count technique at 30ºC
· ISO Standard 20128 (2006) Milk products. Enumeration of presumptive Lactobacillus acidophilus on a selective medium. - Colony-count technique at 37ºC.
· ISO Standard 9232 (2003) Yoghurt – Identification of characteristic microorganisms (Lactobacillus delbrueckii subsp bulgaricus and Streptococcus thermophilus)
· MAN, J.C. de, ROGOSA, M. y SHARPE, M. Elisabeth (1960) A medium for the cultivation of lactobacilli. J. Appl. Bact.; 23:130.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).