

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

Final pH 5.7 ±0.2 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 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 have contaminants, which required 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 CO₂.

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 CO₂ 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 is used for selective enumeration of *Lactobacillus acidophilus* in dairy products. Without the additives but with the pH adjusted to 5.4 can be used for enumeration of *Lactobacillus bulgaricus* in yoghurt.

NOTE: After a long static storage the product tends to cake and compacted without affecting their quality. Its powder fluidity can be recovered with a strong and vigorous shaking the capped container.

Quality control

Incubation temperature: 30 ±1°C

Incubation time: 72 ± 3h

Inoculum: Practical range 100 ±20 CFU. Min. 50 CFU (productivity)/10³-10⁶ CFU (selectivity), according to ISO 11133:2014.

Microorganism

Growth

Remarks

<i>Lactobacillus sakei</i> ATCC® 15521	Productivity > 0.70	Incubate in a 5% CO ₂ atmosphere
<i>Lactococcus lactis</i> ATCC® 19435	Productivity > 0.70	Incubate in a 5% CO ₂ atmosphere
<i>Pediococcus pentosaceus</i> ATCC® 33316	Productivity > 0.70	Incubate in a 5% CO ₂ atmosphere
<i>Bacillus cereus</i> var. <i>mycoides</i> ATCC® 11778	Inhibited	-
<i>Escherichia coli</i> 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 Standard 192 (2006) Enumeration of presumptive *Lactobacillus acidophilus* on a selective medium. - Colony-count technique at 37°C.
- 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 30°C
- ISO Standard 20128 (2006) Milk products. Enumeration of presumptive *Lactobacillus acidophilus* on a selective medium. - Colony-count technique at 37°C.
- 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

Keep tightly closed, away from light, in a dry place (4-30 °C).