**MORETON BAY COLLEGE**

**YEAR 10 TECHNOLOGY & APPLIED SCIENCE – MICROBIOLOGY EEI – COURTESY OF ADAM DELROY**

**Downloaded from seniorbiology.com/eei.html**

For the lactobacillus experiment the following nutrient medium was found to work well (whereas others didn’t work at all).

**Material Safety Data Sheet**

* [Click here to download Material Safety Data Sheets for this product.](http://www.oxoid.com/UK/blue/msds/getMSDS.asp?ph=3&pr=CM0361&sec=&org=82&c=UK&lang=EN)

**Organisms**

Organisms this product works with:

* [Lactobacilli](http://www.oxoid.com/UK/blue/orgbrowse/orgbrowse.asp?ph=2&sec=3&orgListBox=82&c=UK&lang=EN)
* [Leuconostoc](http://www.oxoid.com/UK/blue/orgbrowse/orgbrowse.asp?ph=2&sec=3&orgListBox=88&c=UK&lang=EN)
* [Pediococcus](http://www.oxoid.com/UK/blue/orgbrowse/orgbrowse.asp?ph=2&sec=3&orgListBox=105&c=UK&lang=EN)
* [Streptococci](http://www.oxoid.com/UK/blue/orgbrowse/orgbrowse.asp?ph=2&sec=3&orgListBox=133&c=UK&lang=EN)

**For this Organism**

Other products used in the isolation of [Lactobacilli](http://www.oxoid.com/UK/blue/orgbrowse/orgbrowse.asp?ph=2&sec=3&orgListBox=82&c=UK&lang=EN):

* [CM0113
Tomato Juice Agar](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0113&org=82&c=UK&lang=EN)
* [CM0359
MRS Broth](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0359&org=82&c=UK&lang=EN)
* [CM0361
MRS Agar](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0361&org=82&c=UK&lang=EN)
* [CM0627
Rogosa Agar](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0627&org=82&c=UK&lang=EN)
* [CM0651
Universal Beer Agar](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0651&org=82&c=UK&lang=EN)
* [CM0657
Orange Serum Agar](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0657&org=82&c=UK&lang=EN)
* [CM0777
Raka-Ray Agar Base](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0777&org=82&c=UK&lang=EN)
* [CM0785
M17 Agar](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0785&org=82&c=UK&lang=EN)
* [CM0817
M17 Broth](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0817&org=82&c=UK&lang=EN)
* [CM1153
MRS (ISO) AGAR (DE MAN, ROGOSA and SHARPE)](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM1153&org=82&c=UK&lang=EN)
* [SR0021
Lactic Acid 10%](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=SR0021&org=82&c=UK&lang=EN)
* [SR0222
0.1% Cycloheximide Solution](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=SR0222&org=82&c=UK&lang=EN)
* [PO0231
MRS AGAR](http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=PO0231&org=82&c=UK&lang=EN)

**Dehydrated Culture Media**

**MRS AGAR (DE MAN, ROGOSA, SHARPE)**

**Code:** CM0361

*A solidified version of MRS Broth for the culture of `lactic acid bacteria’.*

|  |  |
| --- | --- |
| **Typical Formula\***  | **gm/litre**  |
| Peptone  | 10.0  |
| `Lab-Lemco’ powder  | 8.0  |
| Yeast extract  | 4.0  |
| Glucose  | 20.0  |
| Sorbitan mono-oleate  | 1ml  |
| Dipotassium hydrogen phosphate  | 2.0  |
| Sodium acetate 3H2O  | 5.0  |
| Triammonium citrate  | 2.0  |
| Magnesium sulphate 7H2O  | 0.2  |
| Manganese sulphate 4H2O  | 0.05  |
| Agar  | 10.0  |
| pH 6.2 ± 0.2 @ 25°C |   |

**\* Adjusted as required to meet performance standards**

**Directions**Suspend 62g in 1 litre of distilled water. Boil to dissolve the medium completely. Dispense into tubes, bottles or flasks and sterilise by autoclaving at 121°C for 15 minutes.

**Description**The MRS formulation was developed by de Man, Rogosa and Sharpe1 to replace a variable product (tomato juice) and, at the same time, provide a medium which would support good growth of lactobacilli in general, even those strains which showed poor growth in existing media. MRS medium is superior to the tomato juice medium of Briggs2 and the meat extract tomato juice medium of de Man. It gives more profuse growth of all strains of *lactobacilli*, especially the difficult and slow growing strains of *Lactobacillus brevis* and *Lactobacillus fermenti.*

MRS Agar and Broth were designed to encourage the growth of the `lactic acid bacteria’ which includes species of the following genera: *Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc*. All these species can produce lactic acid in considerable amounts. They are Gram-positive, catalase and oxidase negative and are fastidious in their nutritional requirements. Growth is enhanced considerably by microaerobic conditions. Generally the `lactic acid bacteria’ show delayed growth and smaller colony size than other micro-organisms. They may be overgrown in non-selective media, especially if incubation is required for 2-4 days.

MRS medium is selective for lactobacilli but some growth of leuconostocs and pediococci may occur. Selectivity can be altered by pH adjustment. Lactobacilli will tolerate lower pH levels than streptococci (pH 5.0-6.5) with pediococci and leuconostocs growing best within this range. Inhibitors of the main groups of competitor microflora include thallous acetate, sodium acetate, sorbic acid, acetic acid, sodium nitrite, cycloheximide and polymyxin. These substances can be used at varying concentrations and combinations but, inevitably, a compromise has to be reached between selectivity and productivity of the organism(s) sought3. MRS Agar with sorbic acid has been described3,4. This is MRS medium with its pH reduced to 5.7 and 0.14% sorbic acid added (equating to 0.2% potassium sorbate).

An evaluation of media for selective enumeration of *Lactobacillus acidophilus* and *Bifidobacterium* species showed that minor adjustments to the basic formula of MRS Agar can readily be made to optimise its performance for determining the content of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in the presence of other lactic acid bacteria which are present in yoghurt5.

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

**Technique**1. Products to be examined for *lactobacilli* content are macerated or diluted in a diluent such as quarter-strength Ringer solution, and further dilutions are made in MRS Broth.
2.  Diluted sample in 1ml volumes are added to sterile dishes, and molten MRS Agar (45°C) is poured into the dish and mixed thoroughly.
3. When the medium has set, another layer of uninoculated MRS Agar is poured over the surface to produce a layer-plate.
4. Plates are incubated as described below. It is important that adequate moisture vapour is present in the atmosphere above the agar because drying of the plates during incubation will concentrate the selective factors on the surface and make the medium inhibitory. The presence of carbon dioxide stimulates growth and plates should be incubated in an atmosphere of 5% CO2.

**Incubation method**

|  |  |
| --- | --- |
| 42°C thermophilic:  | 2 days |
| 35°C mesophilic: | 2 days |
| 30°C + 22°C mesophilic-psychrotrophic  | 2+1 days |
| 25°C psychrotrophic:  | 3 days |

**Incubation carried out under anaerobic or microaerophilic conditions**To identify the presumptive Lactobacillus colonies, select isolated colonies on the agar medium. Stain a smear from each  and pick off into MRS Broth (CM0359), indivdually. An advantage of this broth is that any other micro-organisms, originally lying dormant in the selective agar, are not given the opportunity to multiply, as may occur in a non-selective broth. Incubate the broths at temperatures and times similar to those used for the MRS Agar; they can then be examined microscopically and further sub-cultured to MRS Agar for subsequent confirmation and identification of species.

**Storage conditions and Shelf life**Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared plates at 2-8°C.

**Appearance**Dehydrated medium: Dark straw coloured powder
Prepared medium: Amber coloured gel

**Quality control**

|  |  |
| --- | --- |
| **Positive control:** | **Expected results** |
| *Lactobacillus gasseri* ATCC® 19992 \* | Good growth; pale straw coloured colonies |
| **Negative control:** |   |
| Uninoculated medium  | No change  |

**\* This organism is available as a Culti-Loop®**

**References
1.** de Man J. C., Rogosa M. and Sharpe M. Elisabeth (1960) *Appl. Bact*. 23. 130-135.
**2.** Briggs M. (1953) *J. Dairy Res*. 20. 36-40.
**3.** Reuter G. (1985) *Intern. J. Food Microbiol*. 2. 55-68.
**4.** ISO/TC 34/SC 6/WG 15, No.3 and 5 (1984) *Enumeration of Lactobacteriaceae in meat and meat products*.
**5.** Lankaputhra W.E.V., Shah N.P. and Britz M.L. (1996) *Food Australia* 48. 113-118.

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