02538 Raka Ray Agar, Base (Lactic Acid Bacteria Selective Agar, Base)

A medium for the selective isolation of lactic acid bacteria from beer and brewing processes.

Composition:

Ingredients	Grams/Litre
Yeast extract	5.0
Casein enzymic hydrolysate	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	5.0
Glucose	5.0
Betaine hydrochloride	2.0
Diammonium hydrogen-citrate	2.0
Potassium aspartate	2.5
Potassium glutamate	2.5
Magnesium sulphate	2.0
Manganese sulphate	0.66
Monopotassium phosphate	2.0
N-acetyl glucosamine	0.5
Agar	17.0
Final pH 5.4 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C. Prepared medium appears light yellow coloured, clear to slightly opalescent gel.

Directions:
Suspend 38.5 g in 500 ml of distilled water. Bring to the boil and dissolve the medium completely. Distribute into tubes or bottles and sterilize by autoclaving at 121°C for 15 minutes. Cool to 50-55°C and add contents of 1vial of lactic acid bacteria selective supplement (14121). Additionally 3.5 ml of Cycloheximide solution (18079) can be added (not required). Mix well and pour into sterile petri dishes or dispense as desired.

Inoculation:

Surface Inoculation
Spread 0.1ml of the sample on the agar plates or the specimen is filtered through filter membrane and the membrane is placed on agar plate. Incubate at 25-30°C under anaerobic conditions.

Overlay Technique
4ml sterile molten Raka-Ray Agar is given into a tube which is in water bath at 55°C. 1ml of sample is added to the tube. Mix well and immediately pour the content into a petri dish containing 15- 20ml of solid Raka-Ray Agar to get a optimal distribution of colonies. Incubate under anaerobic conditions at 25-30°C. For further testing it is easy to pick a colony out of the thin agar layer.

Incubation:
An incubation period of 4 days is generally sufficient. The test strains need no longer than 24 hours incubation but slower growing organisms may require up to 7 days.
Depending on the species of lactic acid bacteria a semi-anaerobic atmosphere may be recommendable.

Principle and Interpretation:
Raka-Ray Agar, was developed of Saha, Sondag and Middlekauff for the detection of lactic acid bacteria in beer and brewing processes (1). The European Brewing Convention (EBC) and the American Society of Brewing Chemists (ASBC) recommend using this Agar (2, 3). Diverse members of the family of Lactobacillaceae are important spoilage organisms in the brewing process. This medium was optimised to meet the natural requirements of the Lactobacillaceae. The original Medium Raka-Ray Medium No. 3 (1) was developed to enable brewers to monitor in-process beer quickly and accuarately for a wide range of organisms including pediococci. Originally diverse combinations of stimulating agents were added to Universal Beer Agar to get an optimized media with improved colony size, colony numbers and incubation time. This was then the base for Raka-Ray Medium No. 3 (4).
Van Keer et al. found that Raka-Ray Medium No. 3 yielded the highest colony count of divers media and allowed the enumeration of the greatest number of strains. 30 strains of Lactobacillus have been taken from different origins and was incubated 48 hours under semi-anaerobic conditions (5).

Casein enzymic hydrolysate, liver concentrate, yeast extract provide carbon, nitrogen, amino acids, minerals, vitamins, trace elements and other essential nutrients for growth. Potassium aspartate and potassium glutamate are additional sources of amino acids. Maltose is to detect lactobacilli, which cannot utilise glucose as a carbon source (6) and fructose is the carbon source of *Lactobacillus fructivorans* (5). Glucose is needed as the fermentative energy source for the pediococci (7). Sorbitan mono-oleate (in supplement 14121) act as a stimulant for lactic acid bacteria in general (4). Also, N-acetyl glucosamine, liver concentrate yeast extract and betaine hydrochloride are used as growth stimulating agents. Diammonium hydrogen-citrate and Monopotassium phosphate are the buffering agents while Magnesium and Manganese are important trace elements for Lactobacillaceae. The addition of 3 g/l of phenylethanol (in 14121) inhibits Gram-negative organisms, 5 mg/l amphotericin B (in 14121) and 7mg/l of cycloheximide to inhibit yeasts and moulds.

Cultural characteristics after 18-48 hours at 25-30°C

Organisms (ATCC)	Growth
Leuconostoc mesenteroides (8293)	+++
Lactobacillus acidophilus (11506)	+++
Lactobacillus bulgaricus (11842)	+++
Lactobacillus casei (7469)	+++
Lactobacillus leichmannii (7830)	+++
Lactobacillus plantarum (8014)	+++
Lactobacillus fermentans (9338)	+++
Escherichia coli (25922)	-/+
Sacch. cerevisae (9763)	-

References:

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4. B. Mauld, H. Seidel Brauwissenschaft 24. 105 (1971)
5. C. Van Keer, L. Van Melkebeke, W. Vertriest, G. Hoozee, E. Van Schoonenberghe, J. Inst. Brewing 89. 361-363 (1983)
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Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.