

Technical Data

Lactic Acid Bacteria Selective Agar Base

M1072

Intended Use:

Recommended for selective isolation of lactic acid bacteria.

Composition**

Ingredients	Gms / Litre
Yeast extract	5.000
Tryptone	20.000
HL concentrate#	1.000
Maltose	10.000
Fructose	5.000
Dextrose (Glucose)	5.000
Betaine hydrochloride	2.000
Diammonium hydrogen citrate	2.000
Potassium aspartate	2.500
Potassium glutamate	2.500
Magnesium sulphate	2.000
Manganese sulphate	0.660
Potassium dihydrogen phosphate	2.000
N-acetyl glucosamine	0.500
Agar	17.000
Final pH (at 25°C)	5.4±0.2

^{**}Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.58 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add contents of 1 vial of Lactic supplement (FD055). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactic Acid Bacteria Selective Agar Base is based on the formula of Saha, Sondag and Middlekauff for the detection of lactic acid bacteria in beer and brewing processes (7). It is recommended by European Brewing convention (EBC) and the American Society of Brewing Chemists for isolation of Lactobacilli (2, 6). The family *Lactobacillaceae* has members that are important spoilage organisms in the brewing process.

The original medium viz. Raka-Ray Medium (7) was developed to enable brewers to monitor in-process beer quickly and accurately for a wide range of organisms including pediococci. Further studies towards optimization of conditions of growth factors led to the modification of Raka Ray medium with the addition of sorbitan mono-oleate to stimulate growth of lactic acid bacteria and incorporation of sugars such as fructose as an essential carbohydrate source for *Lactobacillus fructivorans* and maltose for lactobacilli as it lacks the ability to metabolize glucose.

Tryptone provides the nitrogenous compounds, potassium aspartate and potassium glutamate are additional sources of the respective amino acids while diammonium hydrogen citrate buffers the medium.

The addition of phenylethanol and cycloheximide in the supplement (FD055) make the medium selective for the isolation of lactic acid bacteria in beer. Phenylethanol inhibits gram-negative organisms, while yeasts are inhibited by cycloheximide. Polysorbate 80 or sorbitan monooleate (in FD055), HL concentrate, yeast extract and N-acetyl glucosamine act as growth stimulating agents. Fructose is the essential carbohydrate source for *Lactobacillus fructivorans* (8), maltose helps in detection of lactobacilli which cannot utilize glucose (5) whereas glucose is utilized by pediococci (1).

Type of specimen

Brewery samples

Specimen Collection and Handling

Inoculate around 0.1 ml beer sample onto Lactic Acid Bacteria Selective Agar Base plates. Spread the beer sample and overlay with 4 ml of M1072. Incubate at 25-30°C under anaerobic conditions. After use, contaminated materials must be sterilized by autoclaving before discarding.

[#] Equivalent to Liver concentrate

HiMedia Laboratories Technical Data

Warning and Precautions:

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% Agar gel.

Colour and Clarity of prepared medium

Dark amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 7.7% w/v aqueous solution at 25°C. pH: 5.4±0.2

pH

5.20-5.60

Cultural Response

Cultural characteristics observed under anaerobi condition, with added Lactic Supplement (FD055), after an incubation at 25-30°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus acidophilus ATCC 11506	50-100	good-luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	good-luxuriant	>=50%
Lactobacillus fermentans ATCC 9338	50-100	good-luxuriant	>=50%
Lactobacillus brevis ATCC 367	50-100	good-luxuriant	>=50%
Lactobacillus buchneri ATCC 11307	50-100	good-luxuriant	>=50%
Pedicoccus acidilactis ATCC 8042	50-100	none-poor	<=10%
Escherichia coli ATCC 25922 (00013*)	>=104	inhibited	0%
Saccharomyces cerevisiae ATCC 9763 (00058*)	>=104	inhibited	0%

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

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Reference

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- 4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
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- 6. Methods of Analysis of the ASBC (1976) 7th Edition. The Society, St. Paul Mn USA.
- 7. Saha R.B., Sondag R.J. and Middlekauff J.E. (1974) Proceedings of the American Society of Brewing Chemists, 9th Congress, 1974.
- 8. Van Keer B., Van Melkebeke L., Vertriest W., Hoozee G. and Van Schoonenberghe E. (1983) J. Inst. Brewing 89. 361-363.

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