Lactic Acid Bacteria Selective Agar Base

M1072

Lactic Acid Bacteria Selective Agar Base is recommended for selective isolation of lactic acid bacteria from beer and brewing processes.

Composition**

Ingredients	Gms / Litre
Yeast extract	5.000
Casein enzymic hydrolysate	20.000
Liver concentrate	1.000
Maltose	10.000
Fructose	5.000
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
Monopotassium 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 distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50-55°C and aseptically add contents of 1 vial of supplement (FD055) selective for lactic acid bacteria. Mix well and pour into sterile Petri dishes or dispense as desired.

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 (1). It is recommended by European Brewing convention (EBC) and the American Society of Brewing Chemists for isolation of Lactobacilli (2, 3). The family *Lactobacillaceae* has members that are important spoilage organisms in the brewing process.

The original medium viz. Raka-Ray Medium (1) 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.

Casein enzymic hydrolysate 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), liver concentrate, yeast extract and N-acetyl glucosamine act as growth stimulating agents.

Fructose is the essential carbohydrate source for *Lactobacillus fructivorans* (4), maltose helps in detection of lactobacilli which cannot utilize glucose (5) whereas glucose is utilized by pediococci (6).

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.

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 L actic Supplement (FD055), after an incubation at 25-30°C for 18-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
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	>=10^3	inhibited	0%
Saccharomyces cerevisiae ATCC 9763	>=10^3	inhibited	0%

Storage and Shelf Life

Store dehydrated medium and the prepared medium at 2-8°C.Use before expiry date on the label.

Reference

1. Saha R.B., Sondag R.J. and Middlekauff J.E. (1974) Proceedings of the American Society of Brewing Chemists, 9th Congress, 1974.
2. Methods of Analysis of the ASBC (1976) 7th Edition. The Society, St. Paul Mn USA.
3. European Brewing Convention, EBC Analytical Microbiologica: Part II J. Inst. Brewing (1981) 87. 303-321.
4. Van Keer B., Van Melkebeke L., Vertriest W., Hoozee G. and Van Schoonenberghe E. (1983) J. Inst. Brewing 89. 361-363.
5. Lawrence D.R. and Leedham P.A. (1979) J. Inst. Brewing 85. 119.
6. Coster E. and White H.R. (1951) J. Gen. Microbiol. 37.15.

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