

RAKA-RAY AGAR BASE

CAT Nº: 1061

Selective medium for the isolation of lactic acid bacteria in beer and beer fermentation

FORMULA IN g/l

Tryptone	20.00	Diammonium Hydrogen Citrate	2.00
Maltose	10.00	Magnesium Sulfate 7H ₂ O	2.00
Yeast Extract	5.00	Potassium Phosphate	2.00
Fructose	5.00	Liver Extract	1.00
Glucose	5.00	Manganese Sulfate 4H ₂ O	0.66
Potassium Aspartate	2.50	N-Acetylglucosamine	0.50
Potassium Glutamate	2.50	Cycloheximide	0.007
Betaine Hydrochloride	2.00	Bacteriological Agar	17.00

Final pH 5.4 ± 0.2 at 25°C

PREPARATION

Suspend 77.2 grams of the medium in one liter of distilled water. Add 10 ml of Sorbitan Monooleate. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. DO NOT OVEARTHEAT. Cool to 45-50°C and aseptically add 3 grams of Phenylethanol. Mix well and dispense into plates. The prepared medium should be stored at 8-15°C. The color is toasted.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Caution: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

RAKA-RAY AGAR BASE is a selective medium for the isolation of lactic acid bacteria in beer and brewing processes. It yields very good results in the detection of lactobacilli in the fermentation processes of beer.

These organisms can change the organoleptic characteristics of the beer through their metabolites. Detection is complicated because of the nutritional and environmental requirements of these organisms. For these reasons, several formulations have been described to optimize the medium and obtain good growth.

Higher counts of lactobacilli in comparative tests have been obtained with this medium because it contains growth nutrients and stimulants such as Liver extract, Yeast extract, Tryptone, N-acetylglucosamine and Sorbitan Monooleate. Maltose and Fructose are added as sources of carbohydrates when certain lactobacilli can't use glucose. Selectivity is obtained by adding 3 g/l of Phenylethanol, to inhibit Gram-negative bacteria, and Cycloheximide to inhibit yeasts. Sulfate Salts provide inorganic ions. Betaine hydrochloride is used as a growth stimulating agent. Diammonium hydrogen citrate and Potassium phosphate are buffering agents. Potassium aspartate and Potassium glutamate are additional sources of amino acids. Bacteriological agar is the solidifying agent.

The inoculation can be performed by the direct streaking of the agar surface or by the double-layer pour-plate method. Incubation is carried out at 25 - 30°C in anaerobic conditions for 4 days. Some organisms grow slower and may require 7 or more days. *Lactobacillus fermentans* grow with white-cream colonies.

If the number of colonies on each plate exceeds 300, dilute sample 1:10 in sterile saline and re-test.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium, with the additives added, after incubation at a temperature of 25-30°C and observed after 4-7 days.

Microorganisms	Growth
<i>Lactobacillus fermentans</i> ATCC 9338	Good
<i>Escherichia coli</i> ATCC 25922	Inhibited

BIBLIOGRAPHY

Methods of Analysis of the ASBC (1976) 7th Edition, The Society, St. Paul, Mn. USA.

European Brewing Convention, EBC Analytica Microbiologica: Part II J. Inst. Brewing (1981) 87.303-321.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

