

Raka-Ray Agar Base

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

Practical information

Applications	Categories
Selective isolation	Lactic acid bacteria

Industry: Alcoholic beverages

Principles and 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.

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

Formula in g/L

Glucose	5	Bacteriological agar	17
Cycloheximide	0,007	Magnesium sulfate heptahydrated	2
Maltose	10	Potassium phosphate	2
Tryptone	20	Yeast extract	5
Fructose	5	Manganese sulfate tetrahydrate	0,66
Potassium aspartate	2,5	Potassium glutamate	2,5
Betaine hydrochloride	2	Diammonium hydrogen citrate	2
Liver extract	1	N-acetylglucosamine	0,5

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 OVERHEAT. Cool to 45-50 °C and aseptically add 3 grams of phenylethanol. Mix well and dispense into plates.

Instructions for use

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.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Toasted	5,4±0,2

Microbiological test

Incubation conditions: (25-30 °C / 4-7 days).

Microorganisms

Escherichia coli ATCC 25922
Lactobacillus fermentum ATCC 9338

Specification

Total inhibition
Good growth

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

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.