Raka-Ray No. 3 Medium

Intended Use

Raka-Ray No. 3 Medium is recommended for the isolation of lactic acid bacteria encountered in beer and the brewing process.

User Quality Control

Identity Specifications

Difco™ Raka-Ray No. 3 Medium

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	7.49% solution, soluble in purified water with 1% polysorbate 80 upon boiling. Solution is medium to dark amber, clear to very slightly opalescent.
Prepared Appearance:	Medium to dark amber, clear to slightly opalescent.
Reaction of 7.49% Solution at 25°C:	pH 5.4 ± 0.2

Cultural Response

Difco™ Raka-Ray No. 3 Medium

Prepare the medium per label directions (with the addition of 3 g/L phenylethanol and 7 mg/L cycloheximide, adjusted for potency). Inoculate, overlay with 4 mL of sterile medium and incubate anaerobically at 27-30°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	None to poor
Lactobacillus brevis	367	30-300	Good
Lactobacillus buchneri	11307	30-300	Good
Pediococcus acidilactici	8042	30-300	Good

Summary and Explanation

Spoilage organisms are often seriously detrimental to beer flavor. Lactic acid bacteria including lactobacilli and pediococci, which can cause spoilage, are physiologically very diverse.

Raka-Ray No. 3 Medium was developed from a formulation suggested by Saha, Sondag and Middlekauff,[1] who tested a range of ingredients for their ability to stimulate growth of lactic acid bacteria. Polysorbate 80, liver extract, maltose, N-acetyl glucosamine and yeast extract were found to stimulate growth. Tomato juice, free fatty acids and lyophilized beer solids (all of which are found in several media formulations for lactic acid bacteria) were inhibitory.

In comparative studies using in-process beer samples, Raka-Ray media gave higher colony counts for lactobacilli than Tomato Juice Agar, W-L Differential Agar and Universal Beer Agar, with larger colonies developing after 2-4 days of anaerobic incubation.[1,2]

Raka-Ray No. 3 Medium yields larger lactic acid bacterial colonies than Universal Beer Agar.[3] Raka-Ray No. 3 Medium also suppressed the growth of non-lactic acid, facultative bacteria, such as *Aerobacter aerogenes* and *Flavobacterium proteus* that are often associated with lactic beer spoilage organisms.[3]

Raka-Ray No. 3 Medium is also recommended by the 'European Brewing Congress Analytical Microbiologica' for enumeration of lactobacilli and pediococci.[4] The agar may be made more selective by the addition of 3 g of 2-phenylethanol and 7 mg of cycloheximide dissolved in a small quantity of acetone per liter of medium before autoclaving. Yeasts and gram-negative bacteria are suppressed, facilitating enumeration of the lactic bacterial flora.

Principles of Procedure

Polysorbate 80, liver concentrate, maltose and other sugars, N-acetyl glucosamine and yeast extract stimulate the growth of lactobacilli. The optional addition of cycloheximide provides increased selectivity against yeasts and gram-negative bacteria.

Formula

Difco™ Raka-Ray No. 3 Medium

Approximate Formula* Per Liter		
Yeast Extract	5.0	g
Tryptone	20.0	g
Liver Concentrate	1.0	g
Maltose	10.0	g
Fructose	5.0	g
Glucose	5.0	g
Betaine Hydrochloride	2.0	g
Diammonium Citrate	2.0	g
Potassium Aspartate	2.5	g
Magnesium Sulfate	0.98	g
Manganese Sulfate	0.42	g
Dipotassium Phosphate	2.0	g
N-Acetyl Glucosamine	0.5	g
Potassium Glutamate	2.5	g
Agar	16.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 74.9 g of the powder in 1 L of purified water containing 10 mL polysorbate 80. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. To increase selectivity, add 3 g 2-phenylethanol and 7 mg cycloheximide per liter prior to boiling.
3. Autoclave at 121°C for 15 minutes. Avoid overheating, which will cause a softer medium.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Overlay Technique for Enumeration of Lactic Acid Bacteria

1. Inoculate 0.1 mL of the beer sample onto well-dried plates containing 15-20 mL Raka-Ray No. 3 Medium. Five replicates of each sample are recommended.
2. Spread over the surface of the medium using a sterile glass rod.
3. Overlay the surface with 4 mL of the molten sterilized medium cooled to 50°C.
4. Incubate plates at 27-30°C in an anaerobic (H_2/CO_2) atmosphere.

Expected Results

Lactobacilli are visible after 48 hours incubation as smooth, moist colonies that are 1 mm in diameter. Incubate the medium for a total of 7 days to allow development of slow-growing *Pediococcus* strains.

If the number of colonies on each plate exceeds 300, the sample should be diluted 1:10 in sterile physiological saline and retested.

References

1. Saha, Sondag and Middlekauff. 1974. An improved medium for the selective culturing of lactic acid bacteria. Proceedings of the American Society of Brewing Chemists. 9th Congress, p. 9.
2. VanKeer, Van Melkebeke, Vertriest, Hoozee and Van Schoonenberghe. 1983. J. Inst. Brewing *89*:360.
3. Report of the Technical Subcommittee. 1976. Microbiological Controls. J. Am. Soc. Brewing Chemists *34*:93.
4. European Brewing Congress Analytica Microbiologica. 1981. J. Inst. Brewing *87*:314.

Availability

Difco™ Raka-Ray No. 3 Medium

Cat. No. 218671 Dehydrated – 500 g