Lysine Medium Base

M642

Intended Use:

Recommended for isolation and enumeration of wild yeasts in pitching yeasts.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	44.500
Potassium dihydrogen phosphate	1.780
Magnesium sulphate	0.890
Calcium chloride anhydrous	0.178
Sodium chloride	0.089
Adenine	0.00178
DL-Methionine	0.000891
L-Histidine	0.000891
DL-Tryptophan	0.000891
Boric acid	0.0000089
Zinc sulphate	0.0000356
Ammonium molybdate	0.0000178
Manganese sulphate	0.0000356
Ferrous sulphate	0.0002225
L-Lysine	1.000
Inositol	0.020
Calcium pantothenate	0.002
Aneurine	0.0004
Pyridoxine	0.0004
p-Amino benzoic acid (PABA)	0.0002
Nicotinic acid (Niacin)	0.0004
Riboflavin (Vitamin B2)	0.0002
Biotin	0.000002
Folic acid	0.000001
Agar	17.800
Final pH (at 25°C)	5.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 6.62 grams in 100 ml purified / distilled water containing 1 ml of 50% potassium lactate (FD123). Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C, adjust pH to 5.0 with 10% lactic acid and pour into sterile Petri plates.

Principle And Interpretation

Morris and Eddy (4) described this complex medium for the isolation and enumeration of wild yeasts in pitching yeast in the brewery industry. Walters and Thiselton (5) used a liquid synthetic medium containing lysine as sole nitrogen source and found that many types of yeast utilize lysine. Later Morris and Eddy (4) also formulated solid lysine medium. Most of the *Saccharomyces* strains employed in the brewery industry and other fermentative industries do not use lysine, whereas the wild strains do. Lysine Medium exploits this differential behavior to separate both types of yeasts.

The medium contains vitamins and trace elements, which is necessary to support metabolic activities of yeast. Lysine acts as the sole source of nitrogen, which is utilized by many types of yeast. Morris and Eddy (4) recommended surface inoculation of washed aliquots from the yeast mass; 0.2 ml suspension of 107 cells/ml is the best. Sample is incubated at 25°C and examined daily, enumerating all the colonies that have grown (lysine positive). The degree of contamination is expressed as the number of wild yeast cells per million cells of the original inoculum. The number of cells in the inoculum is important as small number

of cells about 100 to 1000 grow to a limited extent while 10,000 brewing yeast cells provide a direct measure of contaminant wild yeasts (1).

Type of specimen

Brewery sample

Specimen Collection and Handling

For brewery samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

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. Due to nutritional variation certain strain may show poor growth.

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 yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.78% Agar gel.

Colour and Clarity of prepared medium

Yellow to Bluish green clear to slightly opalescent opalescent gel forms in Petri plates

Reaction

Reaction of 6.6% w/v aqueous solution at 25°C. pH : 5.0±0.2

pH

4.80-5.20

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C upto 7 days.

Organism	Growth
Growth Promotion Test	
Pichia fermentans ATCC 10651	luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (2,3).

Reference

1. Fowell R. R., 1965, J. Appl. Bacteriol., 28:373.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. 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.

4. Morris E. O. and Eddy A. A, 1957, J. Inst. Brew. 63(1): 34.

5.Walters L. S. and Thiselton M. R., 1953, J. Inst. Brew. 59:401.

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