Modified WL Nutrient Medium

M2000

Intended Use:

Recommended for cultivation and isolation of microorganisms encountered in brewing and industrial fermentations.

Composition**

Ingredients	Gms / Litre
Yeast extract	4.000
Casitose ▲	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	0.550
Potassium chloride	0.425
Calcium chloride anhydrous	0.125
Magnesium sulphate	0.125
Bromocresol green	0.022
Agar	17.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

▲ - Equivalent to Casein hydrolysate

Directions

Suspend 39.25 grams in 1000 ml purified/distilled water. Heat if necessary, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, to obtain a pH of 6.5, add 1% solution of sodium bicarbonate before sterilization. Mix well and pour into sterile Petri plates.

Principle And Interpretation

WL (Wallerstein Laboratory) media are formulated as described by Green and Gray for the examination of materials encountered in brewing and for industrial fermentations containing mixed flora of yeast and bacteria (1, 2). Bakers yeast counts can be carried out in this medium at a pH 5.5. By adjusting the pH to 6.5, the medium can be used for obtaining counts of Baker's and distillers yeast (5). If desired Durham's tubes can be added to WL Nutrient Broth to study fermentation reactions. Yeast extract serves as a source of trace elements, vitamins and amino acids. Casitose is used as a source of nitrogen, amino acids and carbon. Dextrose (Glucose) is the source of carbohydrate. Buffering of the medium is done by potassium dihydrogen phosphate. Potassium chloride and calcium chloride are essential ions that help to maintain the osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromo cresol green is a pH indicator.

Type of specimen

Microorganisms in brewery

Specimen Collection and Handling:

For fermentation samples follow appropriate techniques for handling specimens as per established guidelines (1,2). 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. Further biochemical identification of organisms is required for confirmation.

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
Ligth yellow to light green homogeneous free flowing powder

Gelling
Firm, comparable with 1.70 % Agar gel.

Colour and Clarity of prepared medium
Bluish green coloured very slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 3.73% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH
6.30-6.70

Cultural Response
Cultural characteristics observed after an incubation for 40-48 hours at 35-37°C for bacteria and at 30 ± 2°C for yeasts.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	good-luxuriant	>=50%
Proteus mirabilis ATCC 25933	50-100	good	40-50%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good	40-50%
Saccharomyces uvarum ATCC 28098	50-100	good	40-50%

Key : *Corresponding WDCM numbers.

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 (3,4).

Reference

1. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 12:43
2. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 13:357
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation- Identification- Maintenance of Medical Bacteria, Vol.1,Williams & Wilkins, Baltimore, Md.

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