Lee's Multi-Differential Agar

Intended Use

Lee's Multi-Differential Agar (LMDA), sometimes referred to Schwarz Differential Agar (SDA), is a medium used to select for and differentiate between bacteria found in beer and the brewery.

Summary and Explanation

LMDA was developed to provide a selective medium to isolate beer spoilage organisms, especially acid-producing bacteria such as *Lactobacillus* and *Pediococcus* which are common in brewery environments. The medium contains nutrients and pH buffers to simulate the environment typically found in beer.

The medium also contains a pH indicator and calcium carbonate, which facilitates the differentiation between acid-producing organisms and environmental bacteria. Common acid-producing such as *Lactobacillus* and *Pediococcus* form distinctive zones of clearing and yellow halos around individual colonies.

Principles

Tomato juice provides essential nutrients and acts as a pH buffer. Yeast extract provides essential nutrients for the growth of bacteria and yeast. Dextrose and peptonized milk provide carbon nutrient sources. A number of vitamins and minerals are added to supplement deficient nutrient sources. Calcium carbonate and bromocresol green act as pH indicators. Agar acts as a solidifying agent.

Physical Appearance

LMDA agar appears in dehydrated form as a homogenous, free flowing powder with a blue green tint.

When prepared, LMDA appears as a homogenous clear solid agar without apparent particulate. The media appears blue-green in color prior to inoculation and incubation.

Storage and Shelf Life

Stored dehydrated media in a cool, dry area not exceeding 30^{o} C until expiration date listed on bottle. Store lid tightly between use. Media is highly hydroscopic and will readily absorb moisture. Discard media if media is not free flowing and if premature solidification has occurred.

Do not hold prepared media above 40^{o} C in excess of 4 hours to prevent deterioration of nutrients.

Store prepared petri dishes between 2-8^{o} C for up to 14 days. It is recommended that petri dishes be stored in a seal container to prevent the loss of moisture leading to desiccation.

Precautions and Safety Information

Refer to the Safety Data Sheet for specific hazards, preparation and disposal instructions.

Sterilize all biohazardous waste prior to disposal.

Directions for Preparation

1. Suspend 85 grams of the dehydrated powder in 1 Liter of purified or deionized water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
    a. To inhibit the growth of brewers yeast, add cycloheximide t a concentration at 0.007 g/L
3. Autoclave at 121° C for 15 minutes.
4. Cool to approximately 45^0 C.
5. While still warm, pour the medium into sterile petri dishes. Regularly swirl liquid medium to ensure calcium carbonate remains in suspension.
6. Cool the prepared plates until solidified. Store until use.

Final pH 5.5 ± 0.2 at 25^o C

Directions for Use

1. Inoculate the prepared LMDA plates with desired sample.
    a. Recommended samples include wort, beer or water. It is not recommended to inoculate directly with yeast slurries.
    b. Directly inoculate with 0.1 – 0.2 mL of liquid or enrich sample using membrane filtration.
2. Incubate aerobically or anaerobically for 5 days to ensure complete recovery of lactic acid bacteria.
3. Interpret growth for beer spoilage organisms.

Interpretation of Results

Reaction with LMDA	Presumptive Organism
Medium yellow-colored colonies with zones of clearing	Lactic/acetic acid bacteria
Translucent colonies with green center and zones of clearing	*Lactobacillus* spp.
White colonies with blue undersides, weak clearing	*Acetobacter* spp.
Small pin-prick colonies with zones of clearing	*Pediococcus* spp.
Smooth, shiny spreading colonies without observed clearing	Coliforms

Cultural Response

Organism	Recovery
Lactobacillus brevis	Good
Pediococcus damnosis	Weak
Acetobacter aceti	Good
Saccharomyces cerevisiae	Good
Enterobacter spp.	None

References

1. Lee, S. Y., Jangaard, N. O., Coors, J. H., Hsu, W. P., Fuchs, C. M., and Brenner, M. W. Proc. Am. Soc. Brew. Chem. 33:18, 1975.
2. American Society of Brewing Chemists Methods of Analysis. Microbiological Control – 5. 2009. http://methods.asbcnet.org/methods/MicrobiologicalControl-5

Availability

Weber Scientific LMDA Medium, 500 grams Cat. No. 3118-17

Visit weberscientific.com or contact info@weberscientitific.com for availability and pricing info.