Lin's Cupric Sulfate Medium

Intended Use

Lin's Wild Yeast Medium is a selective medium used to inhibit the growth of brewers yeast (*S. cerevisiae*) while facilitating the growth of several species of wild yeasts.

Summary and Explanation

This medium was designed to isolate and identify beer spoilage yeast that are commonly found in the brewery environment, without encountering erroneous growth of the house brewing yeast. Copper is known to be toxic to yeast and mold at increasing concentrations. Yeast have variable levels of resistance to copper, with *Saccharomyces cerevisiae* being relatively intolerant.

Cupric sulfate is added to this medium to inhibit the growth of *Saccharomyces* brewers yeast. Wild yeast strains such as *Candida*, *Brettanomyces* and *Dekkara* show resistance to low levels of cupric sulfate and are able to grow on the medium. LCSM is best utilized in combination with other wild yeast media to capture the full range of yeast commonly found within the brewing environment.

Principles

Yeast extract, malt extract, peptone, dextrose and ammonium chloride are added to provide nutrients for yeast growth. Dipotassium phosphate is included as a media buffer. Cupric sulfate acts as an anti-fungal and yeast inhibitor. Agar acts as a solidifying agent.

Physical Appearance

LCSM agar appears in dehydrated form as a homogenous, free flowing powder with a white to beige tint.

When prepared, LCSM appears as a homogenous opaque solid agar without apparent particulate. The media appears white to beige 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 7 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 40 grams of the dehydrated powder in 1 Liter of purified or deionized water. Mix thoroughly.
    a. Cupric sulfate is pre-included in the dry medium. No additional ingredients are needed to prepare the medium.
    b. The cupric sulfate concentration in the prepared medium is approximately 0.55 g/L
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
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.
6. Cool the prepared plates until solidified. Store until use. Plates can be stored for up to 5 days at 4^o C

Directions for Use

1. Inoculate the prepared LCSM plates with desired sample.
    a. For product samples (bright or packaged beer), sterile filter 50-100 mL of sample using 0.45 µm membrane filters. Wash the filter with sterile distilled water and aseptically transfer the filter directly to the surface of the prepared media.
    b. For samples containing greater than 1 million yeast cells per mL, or if a membrane filtration setup is unavailable, pipet 0.2 mL of sample directly onto the surface of the prepared medium. Spread evenly across the surface using a sterile swab, sterile inoculating loop or sterile L-shaped spreader.
2. Incubate aerobically between 25-30^o C for 4-6 days.
3. Interpret growth for beer spoilage organisms.

Interpretation of Results

Wild yeast colonies will be identifiable as distinct opaque colonies. Films or "pin prick" colonies should be disregarded and are usually present in high concentration of house brewers yeast. Presumptive positives should be checked under a microscope to confirm cell morphology is consistent with beer spoilage wild yeast.

Certain strains of *S. cerevisiae var. diastaticus* may grow weakly on LCSM.

Cultural Response

Organism	Recovery
Candida spp.	Luxuriant
Bretanomyces spp.	Luxuriant
Dekkara spp.	Luxuriant
Saccharomyces cerevisiae	Weak
Saccharomyces bruxellensis trois	Weak
Enterobacter spp.	None

References

1. Lin, Y. J. Inst.Brew. "Formulation and Testing of Cupric Sulphate Medium for Wild Yeast Detection". May-June 1981, Vol. 87 p. 151-154.

Availability

Weber Scientific LCSM Agar, 500 grams Cat. No. 3118-08

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