Lin's Wild Yeast 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 *Saccharomyces* wild yeast.

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. The medium uses fuchsin sulfate and crystal violet as anti-fungal agents to inhibit the growth of brewers yeast. The concentration of the anti-fungal agents is low enough to prevent the growth of *S. cerevisiae*, but allows for the growth of other *Saccharomyces* spoilage yeast, as well as some *Brettanomyces*, *Candida* and *Dekkara* species.

The concentration of crystal violet can be adjusted based on the growth o the brewers yeast strain used in the brewery. Higher concentrations of crystal violet will have a greater inhibition of brewers yeast, but may also limit the growth of some wild yeast strains.

Principles

Fuchsin sulfate is used as an anti-fungal agent to prevent the growth of *S. cerevisiae*. 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. Agar acts as a solidifying agent. Crystal violet is added separately as an additional anti-fungal agent.

Physical Appearance

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

When prepared, LWYM appears as a homogenous clear solid agar without apparent particulate. Depending on the concentration of crystal violet added, prepared LWYM appears pink to deep purple in color.

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° 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 40 grams of the dehydrated powder in 1 Liter of purified or deionized water. Mix thoroughly.
2. Add 20 mL of 0.1% crystal violet stock solution to the fully-dissolved liquid medium
    a. The volume of crystal violet can be adjusted to provide greater or lower selectivity. Higher concentrations of crystal violet will provide stronger inhibiting of both brewers yeast and wild yeast strains
    b. The ASBC recommends additions of crystal violet between 1 mL to up to 60 mL of 0.1% crystal violet to 1 L of prepared media.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121° C for 15 minutes.
5. Cool to approximately 45^0 C.
6. While still warm, pour the medium into sterile petri dishes.
7. Cool the prepared plates until solidified. Store until use. Plates can be stored for up to 5 days at 4° C

Directions for Use

1. Inoculate the prepared LWYM 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° 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.

Cultural Response

Organism	Recovery
Candida spp.	Luxuriant
Saccharomyces bayanus	Moderate
Saccharomyces uvarum	Moderate
Saccharomyces cerevisiae	Weak
Saccharomyces cerevsisiae var. diastaticus	Weak
Enterobacter spp.	None

References

1. Lin, Y. Am. Soc. Brew. Chem., Proc. 1974, p. 69. American Society of Brewing Chemists
2. Methods of Analysis. Microbiological Control – 5. 2009. http://methods.asbcnet.org/methods/MicrobiologicalControl-5

Availability

Weber Scientific LYWM Agar, 500 grams Cat. No. 3118-11