STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Differential Media Plates

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A. Scope

This procedure describes how to make differential media plates to determine the number of viable ethanol production strain. This organism is unique in that it generates high amounts of acetylaldehyde. This compound turns plates containing pararosaniline from light pink to dark red in and around the colonies

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material listed in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

Refer to UF Biosafety guidelines and the NIH Guidelines whenever handling biological cultures/genetically modified organisms.

During operations in the laboratory, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Glasses
- Protective Gloves (nitrile, neoprene)

C. Related Documents and SOPs

1. Autoclave SOP-0504
2. ESCO Airstream Horizontal Laminar Flow Clean Bench Manual 2008
3. Cell culture incubator manual
4. Balance Manual
5. MSDS Binder

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D. Preparation/Materials/Equipment

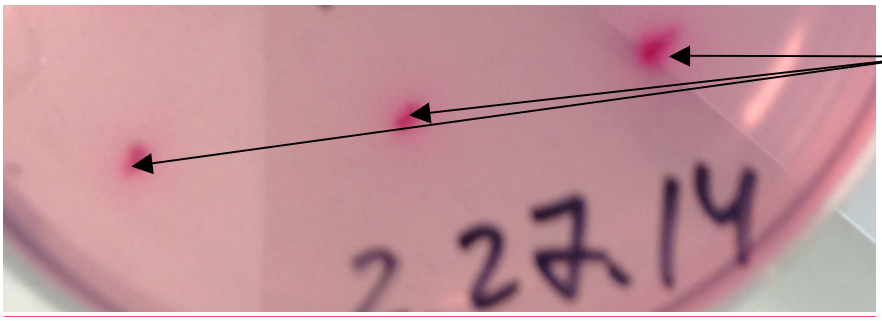
1. Nitrile gloves
2. Autoclave
3. Laminar flow clean bench
4. Cell culture incubator
5. Laboratory balance
6. Stir plate
7. Stir bar
8. Erlenmeyer flasks (1 L)
9. Sterile petri dishes
10. Sterile toothpicks
11. MacConkey agar plates
12. DI water
13. Tryptone
14. Yeast Extract
15. Sodium Chloride
16. Sodium Metabisulfite
17. Pararosaniline
18. 95% Ethanol (v/v)
19. 45 °C water bath with heater circulator
20. Sterile glass beads
21. Sterile pipettes
22. Sterile microcentrifuge tubes
23. Sterile water

E. Detailed Procedure

1. Prepare Luria Bertani/pararosaniline agar
    a. Combine 4 g tryptone, 4 g sodium chloride, 2 g yeast extract, and 4 g agar in a 1 L flask and bring to 400 mL. Mix well using a stir plate and bar.
    b. Autoclave the flask for 30 min in the liquid cycle according to the Autoclave Operation SOP-0504.
    c. Remove the flask from the autoclave and place in a water bath in order to equilibrate to 45 °C.
    d. Bring the flask to the laminar flow clean bench and add 100 mg of sodium metabisulfite.

e. Return to the water bath for 5 minutes. Ensure all of the sodium metabisulfite is dissolved by gently swirling.
f. Prepare the pararosaniline stock.
    i. add 100 mg pararosaniline to a 50 mL glass tube and bring to 40 mL total volume using 95% ethanol (final concentration = 2.5 mg/mL).
    ii. Wrap the tube in foil and store in a dark location. The solution will be good for 1 year.
g. Return the flask to the laminar flow clean bench and add 8 mL of pararosaniline stock to the flask and swirl.
h. Pour ~20-25 mL agar per plate and allow it to set in a dark area as the media is light sensitive. A cardboard box works well for this.
i. Store the plates at room temperature in the dark. They will be good for 1 week and should be a light pink color.

2. Prepare a spread plate of appropriate dilution (30 – 300 colonies per plate) of the sample into MacConkey agar plates.
3. Incubate for 24 h at 30 – 37 °C.
4. Count the colonies obtained in the MacConkey agar plates and record the information.
5. Using sterile toothpicks, pick each colony from the MacConkey agar into the Luria Bertani/pararosaniline agar and incubate at 37 °C for no longer than 2 hours.
    a. A positive result will be a dark pink- red area in and around the colony, spreading away from the colony.
    b. Overnight incubation will result in an unreadable plate as it will be completely red.
6. Record the number of positive colonies and compare to the initial count.



F. Data Archival and Analysis

Record the number of pink-red colonies in the laboratory notebook including the sample name, date, time, vessel and dilution used for plating.