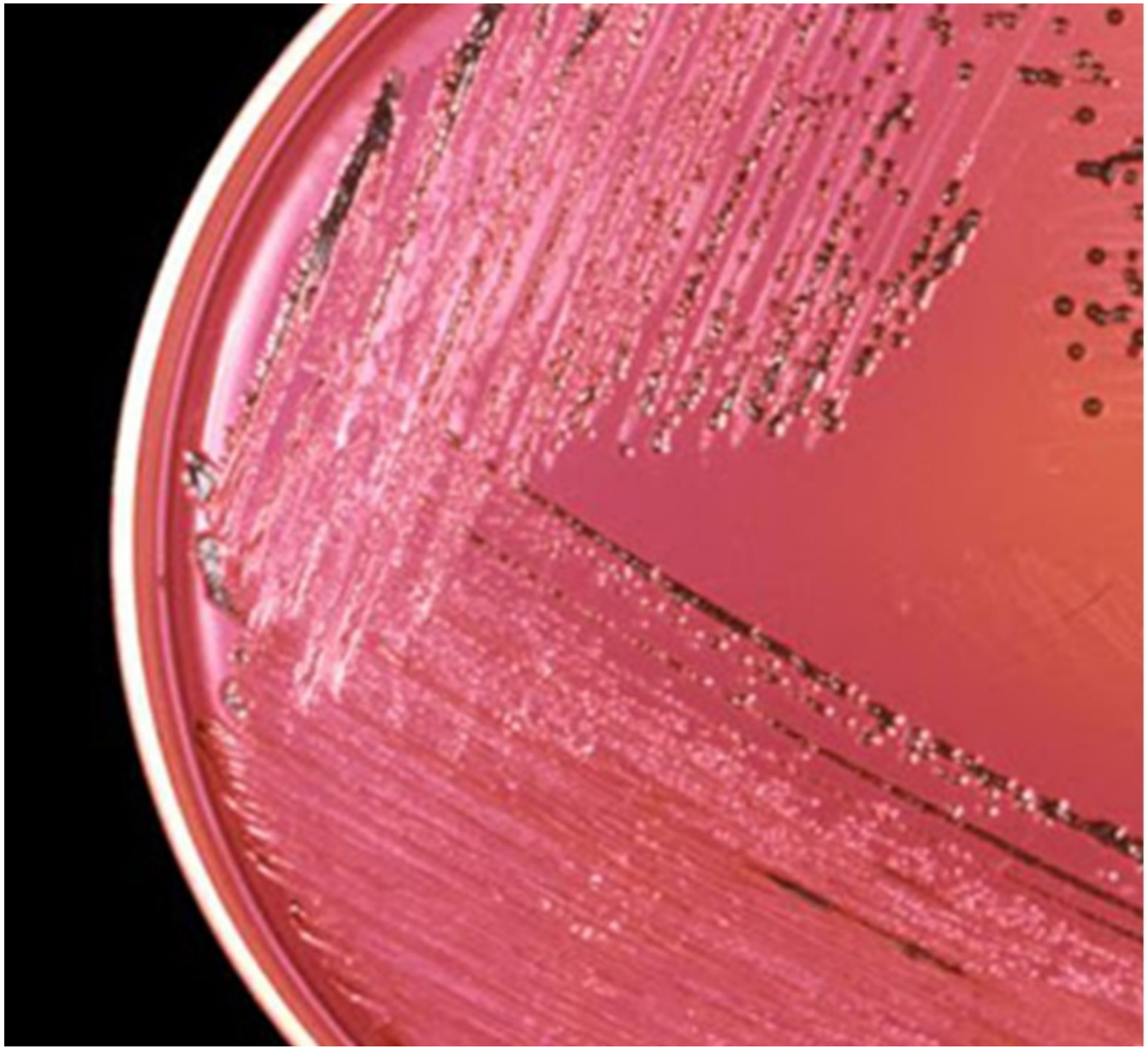

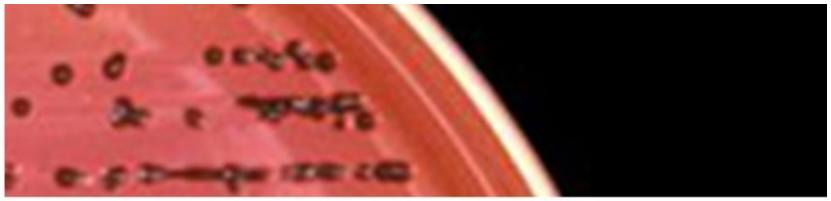

DATE: 11 DECEMBER 2020

2ND PROFESSIONAL YEAR
(VETERINARY MICROBIOLOGY)

TOPIC :

METHODS OF INOCULATION, CULTIVATION OF AEROBIC AND ANAEROBIC BACTERIA

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CULTURE METHODS

- Bacteria are grown (cultured) for identification.

- By suitable procedures they have to be grown individually (isolated) on culture media and thus obtained as pure for study.

Colony :

Macroscopically visible collection of millions of bacteria originating from a single bacterial cell.

History

- Louis Pasteur first to use media – urine or meat broth
- Robert Koch used cooked cut potato – earliest solid medium
- Gelatin – not satisfactory
  - liquefy at 24°C

Culture Methods

Purpose of culture :

- To isolate bacteria in pure cultures.
- To demonstrate their properties.
- To obtain sufficient growth for preparation of antigens & for other tests.
- For typing bacterial isolates.
- To conduct antibiotic sensitivity.
- To estimate viable counts.
- To maintain stock cultures.
- To generate strains of interest
- To carry out recombinant protein expression

Culturing methods

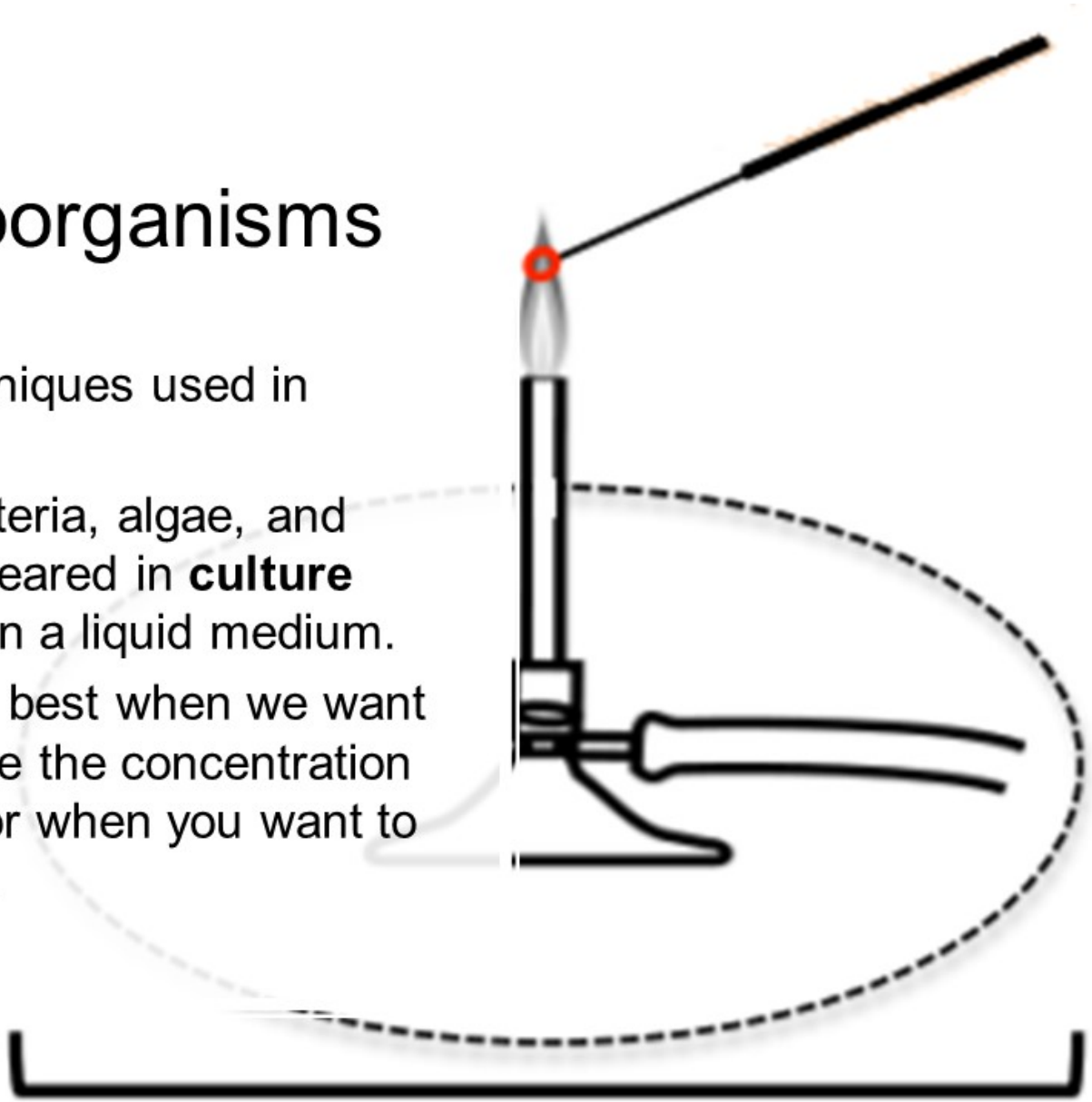
Culturing Microbes

The Five "I's

- **Inoculation:** Producing a pure culture
- **Isolation:** Colony on media, one kind of microbe, pure culture
- **Incubation:** growing microbes under proper conditions
- **Inspection:** Observation of characteristics (data)
- **Identification:** use of data, correlation, to ID organism to exact species

Culturing Microorganisms

- Two basic culture techniques used in microbiology:
  1. ***Liquid culture***: bacteria, algae, and some fungi can be reared in **culture tubes** (**test tubes**) in a liquid medium.
     - *Liquid medium* is best when we want to rapidly increase the concentration of the organism or when you want to grow motile cells.



Culturing Microorganisms

2. ***Culture Plates:*** Liquid medium is solidified using **agar** (agarose) and poured as a thin layer in the bottom of a culture dish (also sometimes called **petri plate**)
   - *Culture plates* are used when you want to test (1) antibiotic sensitivity, (2) estimate culture concentrations from environmental samples, or (3) isolate individual colonies from environmental samples.

Colony – macroscopically visible collection of millions of bacteria originating from a single bacterial cell.

Types of culture methods

INOCULATION IN CULTURE PLATE

- Streak culture or surface plating
- Spread or lawn culture
- Pour plate method

INOCULATION IN TUBES

- Stroke culture
- Stab culture

- Anaerobic methods of culturing bacteria

Culture methods

Streak culture

- Isolation of bacteria in pure culture from clinical specimen

Spread or Lawn culture

- Antimicrobial susceptibility testing (disc diffusion), bacteriophage typing

Liquid cultures

Stroke culture

- To obtain pure growth for slide agglutination; biochemical tests

Stab culture

- Maintenance of stock cultures

Pour-plate culture

- Quantification of bacteria in liquid cultures, urine sample

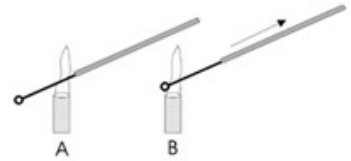
STREAK CULTURE

- **PROTOCOL**
  - One loopful of the specimen is transferred onto the surface of a well dried plate.
  - Spread over a small area at the periphery.
  - The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
  - On incubation, separated colonies are obtained over the last series of streaks.

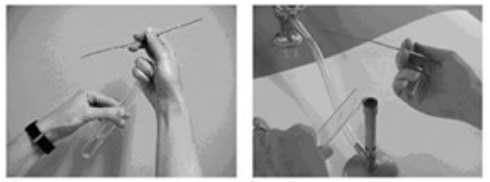
All activities should be under aseptic conditions!!

Inoculation of Culture Plates and Tubes

- ✓ Use either disposable inoculation loops or a metal loop that can be heat sterilized to inoculate plates, slants, and liquid culture tubes.



- ✓ If using a metal loop, be sure to cool the loop by touching the sterile cooled liquid media or the sterile culture plate **before** the placing the loop in your live culture.

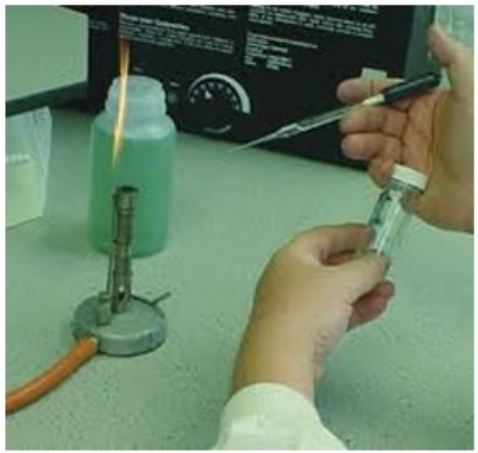


- ☛ Failure to cool the loop will kill your active microbial cultures!

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 1:

i. Remove the culture tube stopper or cap with one (do not set it down) and flame the mouth of the tube to surface sterilize the mouth.



ii. The heated tube surface will generate a thermal current that prevents contamination of the culture.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 2: Place the sterile inoculation loop on working bench without touching any culture.

Step 3: Replace cap/cotton plug on the culture tube with the active microbes and pass inside in the test tube rack.

Step 4: Without pressing the loop down, pick-up a sterile fresh culture tube (*with media with one hand, and remove the cap with the other hand*).

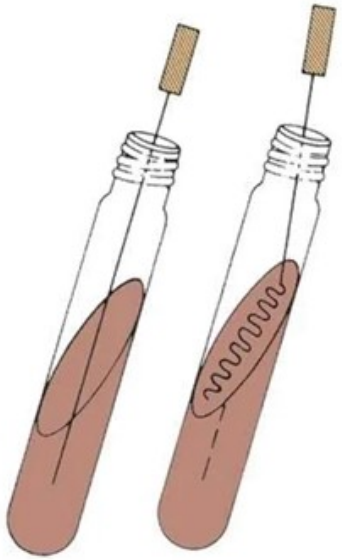
Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 5: Flame the mouth of the culture tube by rotating in circular fashion .

Step 6: Place the inoculation loop loaded with the microbes in the fresh media and swirl the loop in the media to ensure its even dispersal in the media.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 7: While using a solid media slant tube, follow above five steps and then zig-zag the inoculation loop across the slanted surface of the solid media in the tube.



Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 8: Flame the mouth of the newly inoculated culture tube and replace the cap.

Step 9: Place the culture tube back in test tube rack.

Step 10: Repeat until all of the sterile tubes have been inoculated. Use a fresh disposable culture loop for each tube or flame the metal loop after each tube has been inoculated.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 11: Incubate the culture at the recommended temperature (check with your supplier for growth requirements).

Avoid

- incubation at room temperature
- accidental culture of human pathogens.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 12:

i. Dispose of all culture materials in a biohazard bag and sterilize all old cultures before pouring out cultures and washing culture tubes.

ii. Disposable culture dishes should be melted in an autoclave or pressure cooker prior to disposal.

Inoculating Petri Plates

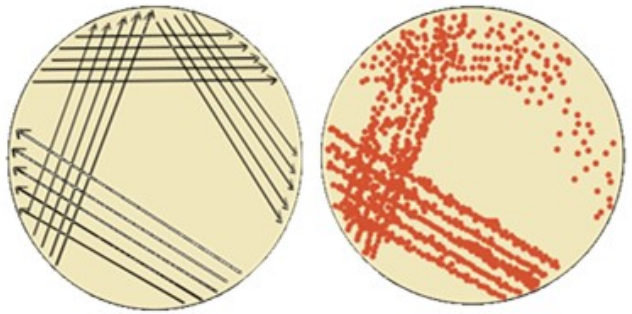
Step 1:Remove the culture tube stopper or cap with one and flame the mouth of the tube to surface sterilize the mouth. The heated tube surface will generate a thermal current that prevents contamination of the culture.

Step 2: Without setting any of the culture materials on the bench, place the sterile inoculation loop in the culture.

Step 3: Replace cap on the culture tube with the active microbes and put it in the test tube rack.

Inoculating Petri Plates

Step 4: Holding the petri dish lid at an 30-45 degree angle, run the inoculating loop from the outside of the plate toward the center in a zig-zag pattern that covers approximately 25% of the plate surface.



Inoculating Petri Plates

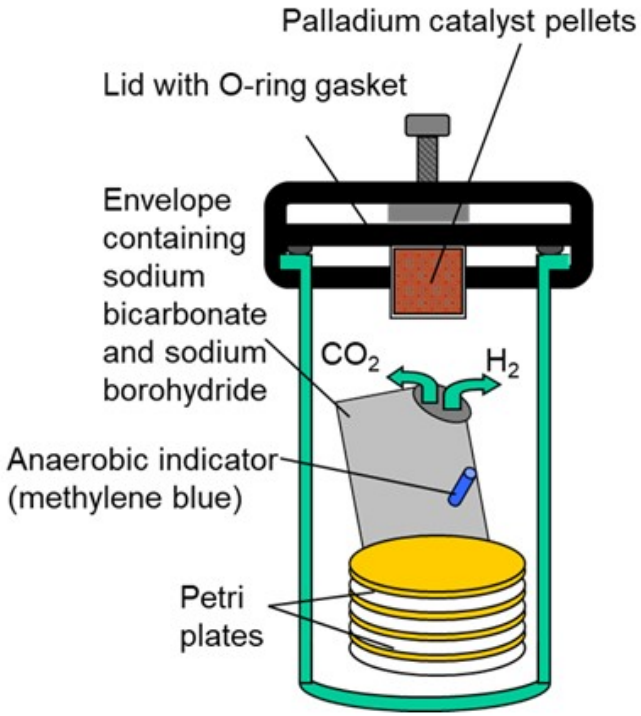
Step 5: Turn the petri plate 90° to the right, dragging the inoculation loop through the last section of the plate, moving from the outside to the inside in a zig-zag motion.

Step 6: Repeat this process twice more until the entire plate . surface is covered.

Anaerobic growth

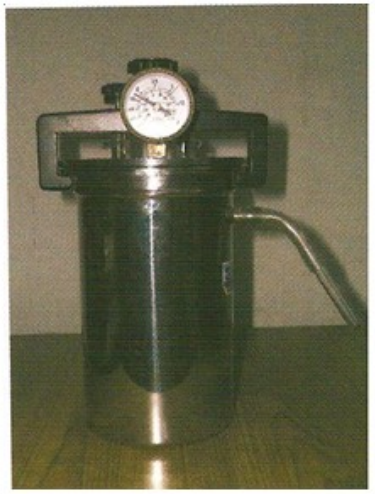
- Reducing media containing thioglycolate to deplete oxygen; cooked meat broth; reducing agent - 1% glucose 0.1% thioglycolate,0.1% ascorbic acid 0.05% cysteine

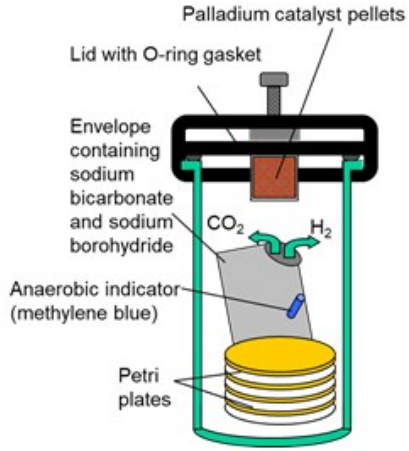
- Anaerobic jar, anaerobic chamber, anaerobic bags/ pouch



DISPLACEMENT AND COMBUSTION OF OXYGEN

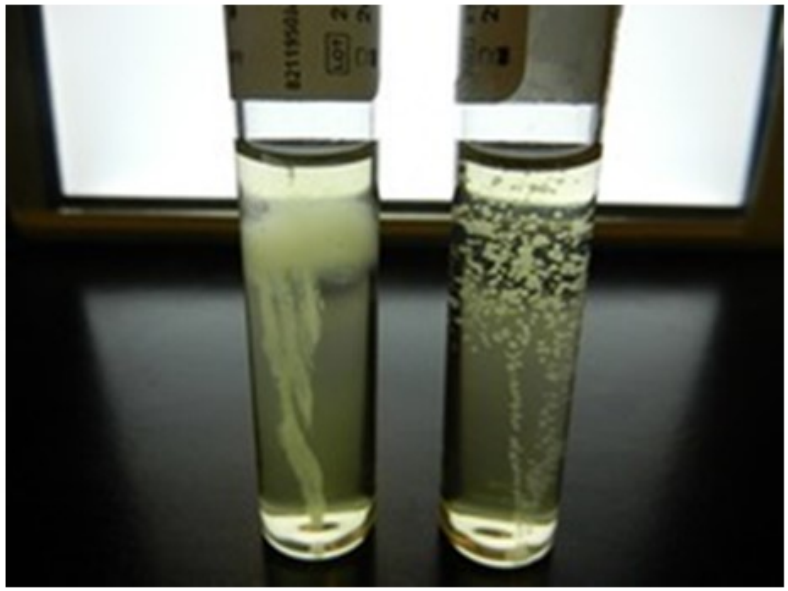
- Most reliable & widely used anaerobic method
- Complete anaerobiosis
- Catalyst – palladinised asbestos
- Commercially available as disposable envelope, containing chemicals which generate H_2 , CO_2 with the addition of water.



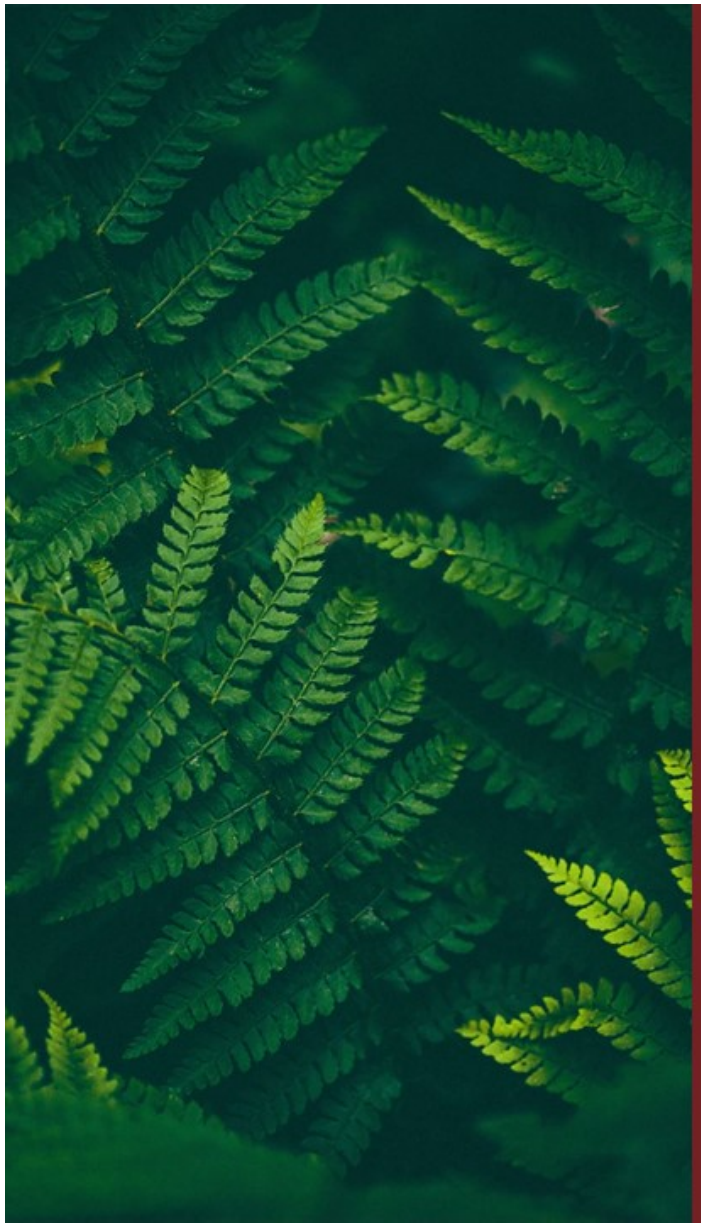


ANAEROBIC CULTURE MEDIA

THIOGLYCOLLATE BROTH



ROBERTSON'S COOKED MEAT MEDIUM



THANKS

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