# Fermentation Lab

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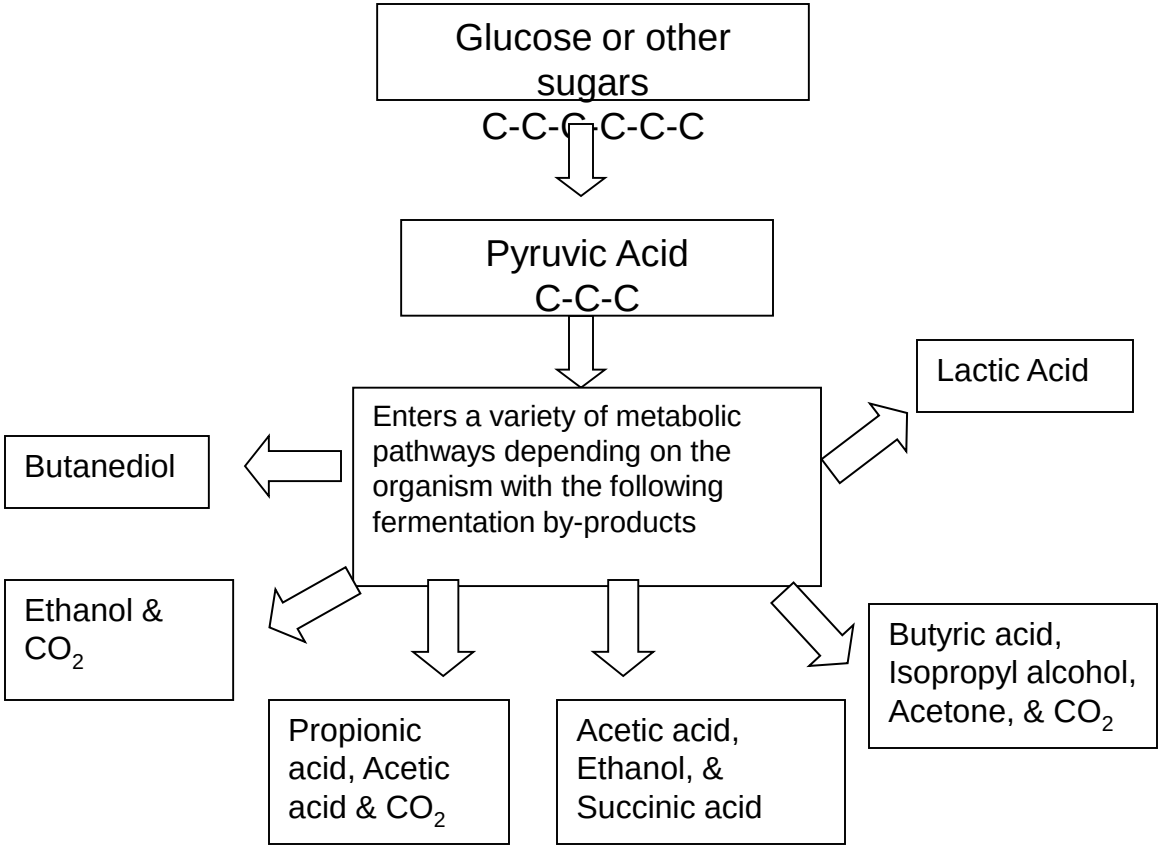


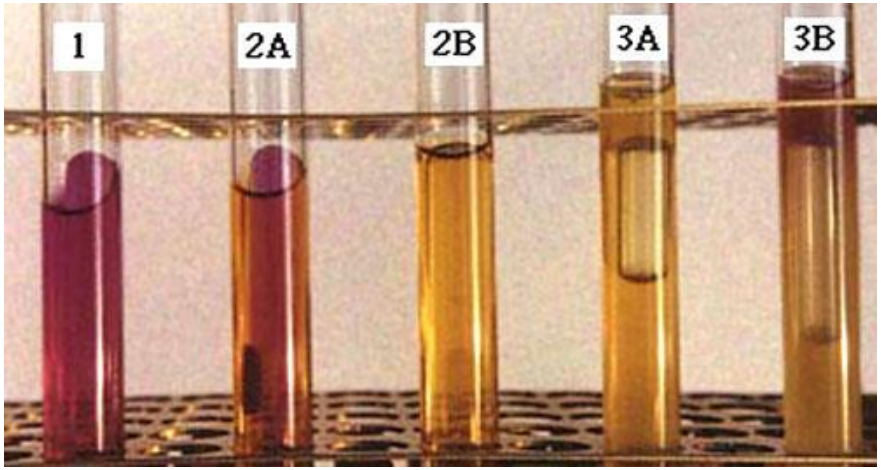
# Media Used For Determination

- ***Fermentation Broth***
  - **Fermentation** of carbohydrates results in the **abundant production of acidic end products**, the presence of which can be **detected by the pH indicator** in the medium.
  - Many organisms produce **gas** – either $CO_2$ alone or a mixture of $H_2$ and $CO_2$. **$H_2$ is insoluble** and is **detected by bubble formation in a Durham tube** placed in the medium.

# Durham Tube

- A Durham fermentation tube is a test tube that has a second inverted tube inside used to capture fermented gas byproducts of bacteria.
- Typically the tube has a pH indicator in it, like phenol red, that will change color when acid is present (in this case to yellow).
- Gases are trapped inside the Durham tube, indicating the production of gas.

# Possible Results




**Tube 1: No fermentation**. The pH indicator remains red. **There can still be growth** due to the use of amino acids as sources of energy (usually by respiration).

**Tubes 2A and 2B: Fermentation with the production of acid (yellow color) but no gas.** A slight amount of acid is seen in tube 2A, but fermentation is still recorded for this tube.

**Tubes 3A and 3B: Fermentation with the production of acid (yellow color) and insoluble gas (bubble in Durham tube).** Tube 3B shows an alkaline reaction on top; this is simply due to deamination of amino acids whose alkaline reaction has not been over-neutralized by the acid diffusing through the tube from fermentation.

# Your Experiment

- You will use a loop to inoculate each of five different fermentation tubes with your unknown bacterium.
  - Dextrose (D-glucose)
  - Lactose
  - Sucrose
  - Maltose
  - Mannose

# Cont'd

- The tubes will incubate at 30 degrees Celsius.

- Tubes will be scored next week
  - Growth +/- (Turbidity?!)
  - Acid +/- (Yellow vs. red)
  - Gas +/- (Bubble in Durham tube)

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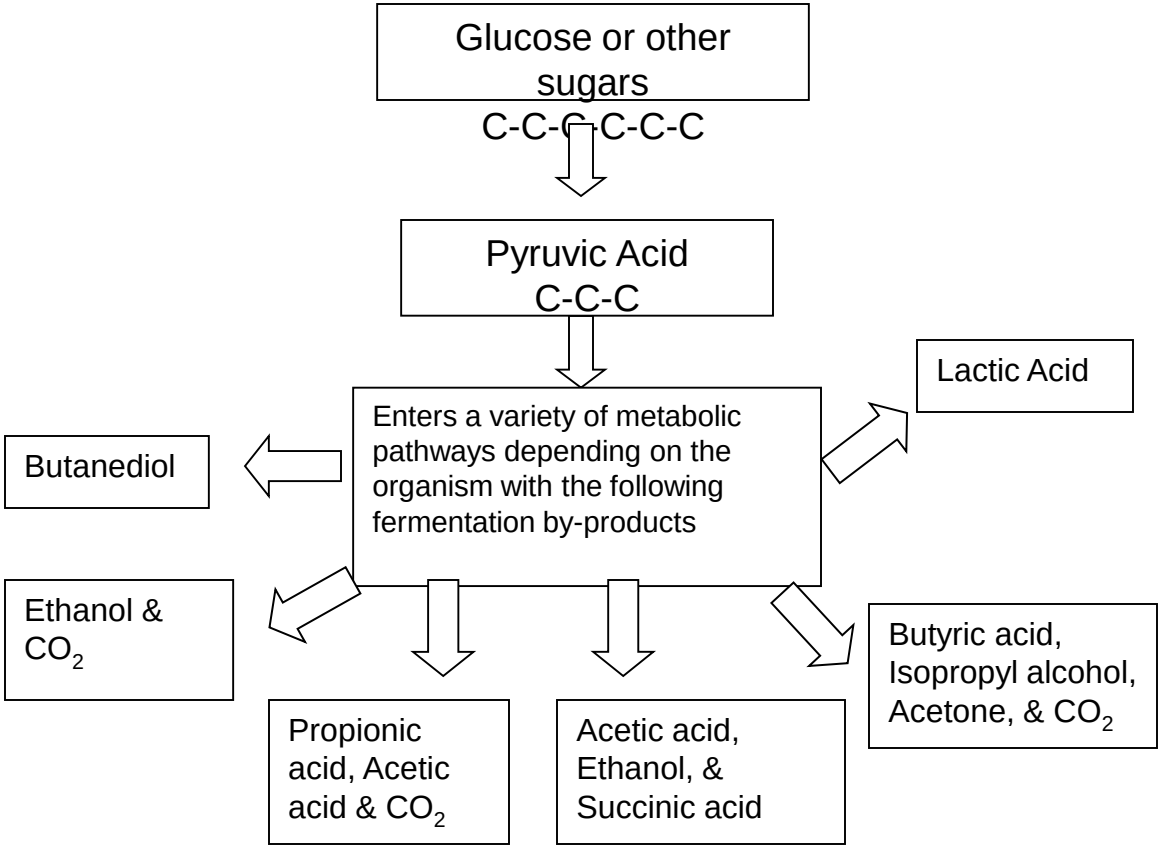

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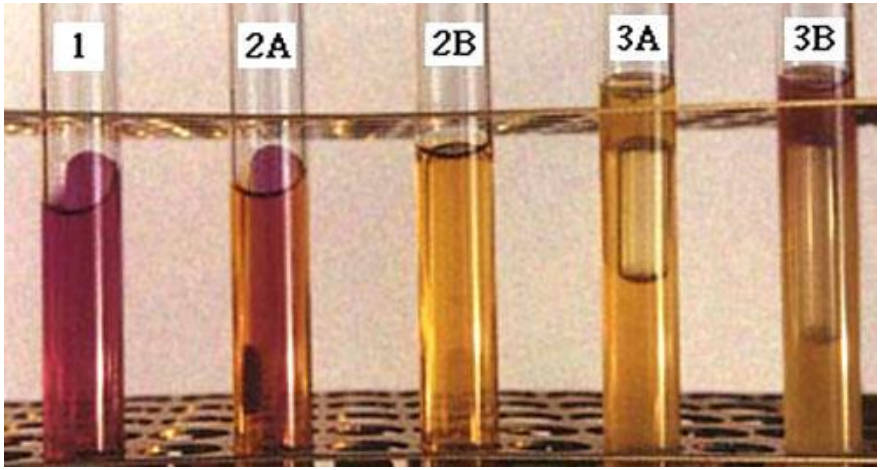


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# Eosin-Methylene Blue Agar Plates Protocol

Created: Saturday, 29 September 2007

**Author**

- Archana Lal
- Naowarat Cheeptham

## Information

### History

Eosin-methylene blue (EMB) agar was first developed by Holt-Harris and Teague in 1916. They used EMB agar to clearly differentiate between the colonies of lactose fermenting and nonfermenting microbes. In the same medium, sucrose was also included to differentiate between coliforms that were able to ferment sucrose more rapidly than lactose and those that were unable to ferment sucrose. Lactose fermenter colonies were either black or possessed dark centers with transparent and colorless outer margins, while lactose or sucrose nonfermenters were colorless. EMB agar was shown to be more sensitive and stable and differentiated between sugar fermenters and nonfermenters faster when compared to other agars.

In 1918, Levine described an EMB agar that differentiated between fecal and nonfecal types of the coli aerogenes group. It also differentiated between salmonellae and other nonlactose fermenters from the coliforms.

Present day Bacto EMB agar is a combination of the EMB agar described by Holt-Harris and Teague and Levine. It contains lactose and sucrose (as described by Holt-Harris and Teague) and also contains Bacto peptone and phosphate (as described by Levine). The two indicator dyes, eosin and methylene blue, are used in a ratio to impart minimum toxicity but provide best differentiation.

### Purpose

Eosin-methylene blue agar is selective for gram-negative bacteria against gram-positive bacteria. In addition, EMB agar is useful in isolation and differentiation of the various gram-negative bacilli and enteric bacilli, generally known as coliforms and fecal coliforms respectively (8). The bacteria which ferment lactose in the medium form colored colonies, while those that do not ferment lactose appear as colorless colonies (1). EMB agar is used in water quality tests to distinguish coliforms and fecal coliforms that signal possible pathogenic microorganism contamination in water samples. EMB agar is also used to differentiate the organisms in the colon-typhoid-dysentery group: Escherichia coli colonies grow with a metallic sheen with a dark center, Aerobacter aerogenescolonies have a brown center, and nonlactose-fermenting

gram-negative bacteria appear pink (5).

**Theory**

EMB agar contains peptone, lactose, sucrose, and the dyes eosin Y and methylene blue; it is commonly used as both a selective and a differential medium. EMB agar is selective for gram-negative bacteria. The dye methylene blue in the medium inhibits the growth of gram-positive bacteria; small amounts of this dye effectively inhibit the growth of most gram-positive bacteria (8). Eosin is a dye that responds to changes in pH, going from colorless to black under acidic conditions. EMB agar medium contains lactose and sucrose, but not glucose, as energy sources. The sugars found in the medium are fermentable substrates which encourage growth of some gram-negative bacteria, especially fecal and nonfecal coliforms. Differentiation of enteric bacteria is possible due to the presence of the sugars lactose and sucrose in the EMB agar and the ability of certain bacteria to ferment lactose in the medium. Lactose-fermenting gram-negative bacteria (generally enteric) acidify the medium, and under acidic conditions the dyes produce a dark purple complex which is usually associated with a green metallic sheen. This metallic green sheen is an indicator of vigorous lactose and/or sucrose fermentation ability typical of fecal coliforms. A smaller amount of acid production, which is a result of slow fermentation (by slow lactose-fermenting organisms), gives a brown-pink coloration of growth. Colonies of nonlactose fermenters appear as translucent or pink (6, 9).

**RECIPE**

As described in the Difco manual (2):

10 g of Bacto peptone
5 g of Bacto lactose
5 g of Bacto sucrose
2 g of dipotassium phosphate
13.5 g of Bacto agar
0.4 g of Bacto eosin Y
0.065 g of Bacto methylene blue
Distilled water t o bring final volume to 1 liter.

Adjust pH to 7.2. Boil to completely dissolve agar. Sterilize in an autoclave for 15 minutes at 15 psi (121oC). Cool to 60oC. If any precipitate is apparent in the medium, disperse by gently swirling before pouring into sterile Petri dishes (1).

EMB agar is commercially available in premixed form from biological supply companies.

**PROTOCOL**

Obtain an EMB agar plate and streak it with the appropriate bacterial culture using the quadrant streak plate method. This will result in the


 

isolation of individual colonies.

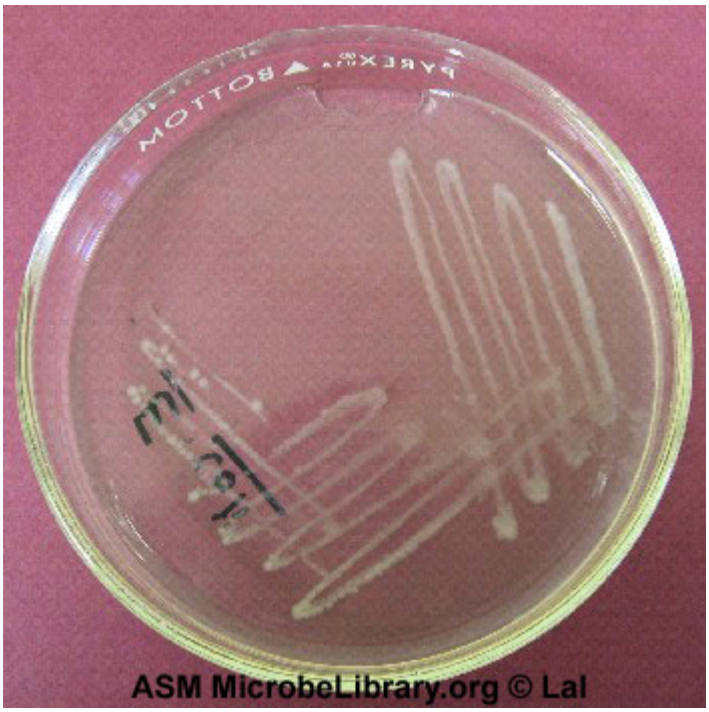


FIG. 1. Nutrient agar inoculated with Escherichia coli (a gram-negative bacterium) demonstrating growth with whitish colonies.

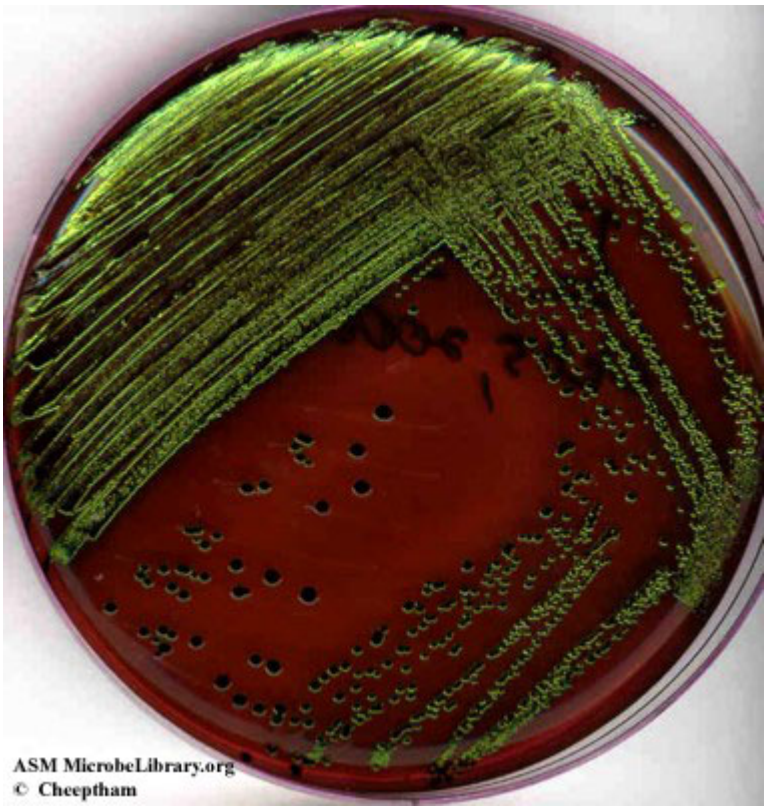

FIG. 2. EMB agar inoculated with Escherichia coli (a gram-negative bacterium) demonstrating growth with green-metallic sheen colonies (for details see the Atlas page).


FIG. 3. Nutrient agar inoculated with Pseudomonas aeruginosa (a gram-negative bacterium) demonstrating growth with whitish colonies.


FIG. 4. EMB agar inoculated with Pseudomonas aeruginosa (a gram-

negative bacterium) demonstrating growth with pinkish mucoid colonies (for details see the Atlas page).

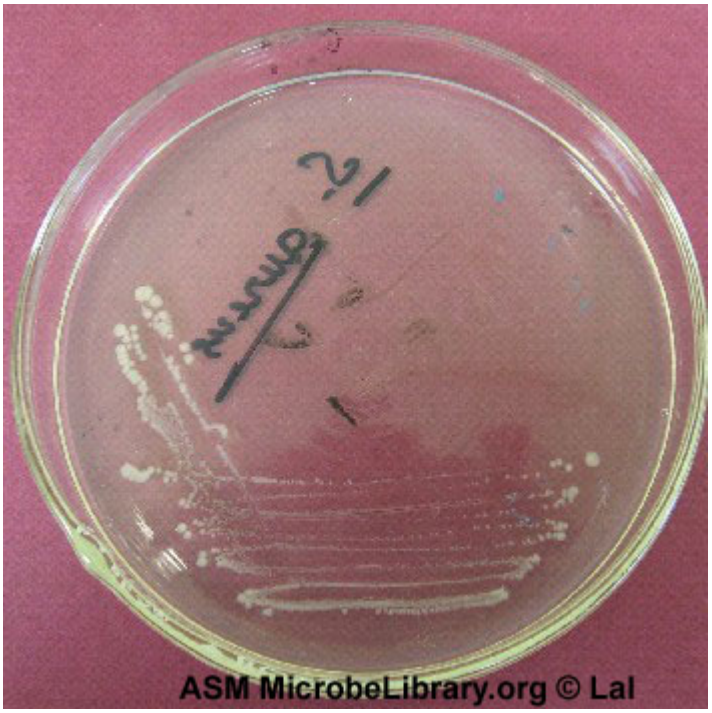


FIG. 5. Nutrient agar inoculated with Staphylococcus aureus (a gram-positive bacterium) demonstrating growth with whitish colonies.

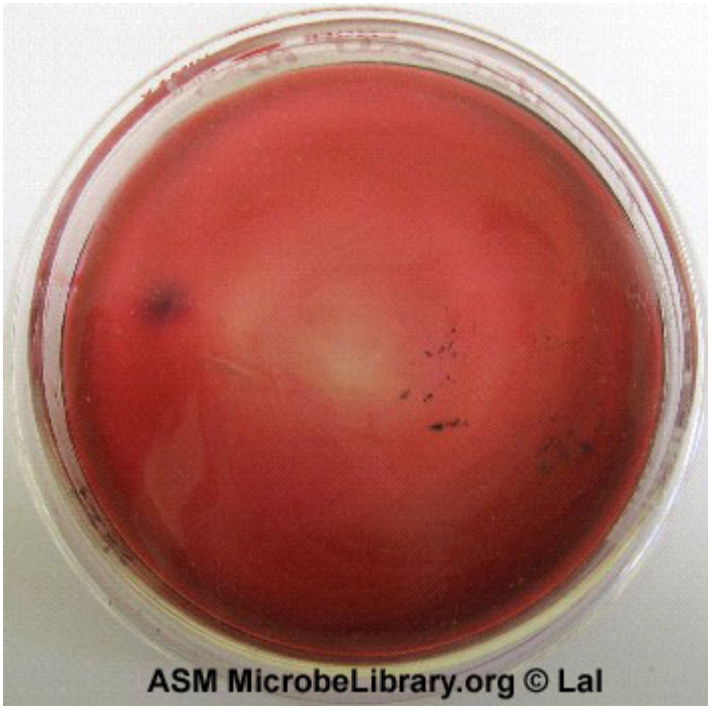


FIG. 6. EMB agar inoculated with Staphylococcus aureus (a gram-positive

bacterium) demonstrating no growth. EMB medium inhibits the growth of gram-positive bacteria.

## SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

## COMMENTS AND TIPS

The concentration of agar may be increased to 5% (by using an additional 3.65 g of agar per 1 liter of medium, refer to the recipe section) to inhibit the spreading of Proteus (1).

If the sucrose-containing EMB medium is used, Proteus colonies will also show the characteristic metallic sheen if they are inhibited from spreading by the higher concentration of agar (1).

Besides being used as a fermentation indicator medium to differentiate gram-negative enteric bacteria, EMB can also be used for testing strains of bacteria for sensitivity to phage. In this case, 5 g of NaCl per liter is to be added into the medium, and the medium is to be made without added sugars to a final concentration of 1% as in the typical EMB. This medium is then designated as EMBO agar (3).

## REFERENCES


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