Phenylethyl Alcohol Agar Protocol

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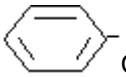
Information

History

Brewer and Lilley (3) developed a selective medium containing phenylethyl alcohol (PEA) that allowed growth of gram-positive organisms, particularly cocci, while inhibiting most gram-negative bacteria and fungi (4, 9).

PEA (benzylcarbinol, $C_6H_5CH_2$-CH_2OH,  CH_2CH_2OH), found in volatile oils of many flowers such as roses, is colorless and soluble in water. With a boiling point between 219 and 222°C, it can be synthesized by the reduction of ethyl phenylacetate with sodium in absolute alcohol (9). Brewer observed a selective phenomenon when 5 ml of 1:30 PEA in acetone was placed on a pad on a petri dish lid covering an agar inoculated with various microorganisms. He found that under this situation, gram-negative organisms (*Pseudomonas aeruginosa* and*Proteus*) did not overgrow while the gram-positive organism (*Staphylococcus aureus)* grew abundantly (4).

Lilley and Brewer researched the optimum concentration of PEA for differential inhibition and found it to be 0.25% (9). In this study, PEA was also found to show fungistatic activity. When 16 molds and yeasts were tested, only*Candida albicans* grew on Sabouraud's medium containing 0.25% PEA. In 1986, Sugita et al. reported that isolation of anaerobic gram-positive cocci from marine animals could be done by using a modified glucose blood liver agar with 0.3% PEA (11).

Lilley and Brewer (9) reported that organisms grown on basic media containing PEA do not change genetically. Organisms grown on media with PEA show normal growth characteristics when subcultured on a medium without PEA.

PEA agar with 5% sheep blood is used in microbiology laboratories to inhibit gram-negative bacteria, specifically*Proteus* species, in specimens containing a mixed bacterial flora (1). Five percent sheep blood is added to the base medium to enhance the growth of anaerobic bacteria. Most gram-positive and gram-negative anaerobes grow on PEA agar medium, especially in mixed culture, and morphology of colonies is similar to that on blood agar plates, however, a longer incubation time is necessary to detect the more slowly growing and pigmented anaerobes (7).

Purpose

PEA agar is a selective medium that is used for the isolation of gram-positive *Staphylococcus* species and*Streptococcus* species from clinical specimens or specimens that contain mixtures of bacterial flora (2). Typically PEA agar is used to inhibit the common contaminants such as *Escherichia coli* and *Proteus* species. PEA agar may be prepared with and without 5% sheep blood supplement. PEA agar with 5% sheep blood is used to isolate most gram-positive and gram-negative anaerobes from enteric samples (7). It is used to inhibit facultative gram-negative rods, preventing *Enterobacteriaceae* from overgrowing the anaerobes and inhibiting swarming of *Proteus* and*Clostridium septicum* (6, 7). PEA agar is used for purulent specimens and when mixed infections are suspected (7).

TABLE 1. Examples of growth of some gram-negative and gram-positive bacteria on PEA agar

Gram reaction	Organism	Growth response	Swarming inhibition
Gram negative	*Escherichia coli*	Inhibited	N/A
Gram negative	*Proteus mirabilis*	Markedly inhibited	Yes, no spreading
Gram negative	*Pseudomonas aeruginosa*	Partially inhibited	N/A
Gram negative	*Salmonella enteritidis*	Inhibited	N/A
Gram negative	*Enterobacter aerogenes*	Inhibited	N/A
Gram positive	*Staphylococcus aureus*	Good	N/A
Gram positive	*Streptococcus pyogene*	Good	N/A
Gram positive	*Streptococcus pnuemoniae*	Good	N/A
Gram positive	*Clostridium perfringens*	Partially inhibited	N/A
Gram positive	*Enterococcus faecalis*	Good	N/A
Gram positive	*Bacillus* sp.	Good	N/A
Gram positive	*Micrococcus luteus*	Good	N/A

Please refer to the PEA Agar Atlas to see the growth patterns.

Theory

PEA agar is a selective medium that permits the growth of gram-positive cocci while inhibiting most gram-negative organisms. PEA acts on gram-negative bacteria by altering their membrane permeability, allowing influx of otherwise blocked molecules, and allowing leakage of large

amounts of cellular potassium that ultimately results in disruption or inhibition of DNA synthesis (6, 8, 9, 10).

RECIPES (1, 2, 12)

PEA agar typical composition (g/liter) (12)

Pancreatic digest of casein	15 g
Papaic digest of soybean meal	5 g
Sodium chloride	5 g
β-Phenylethyl alcohol	2.5 g
Agar	15 g
Distilled water	1.0 liter

Suspend the first five ingredients in 1 liter of distilled water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to dissolve completely. Autoclave the medium at 121°C for 15 minutes at 15 psi. Final pH of the medium should be 7.3 $\pm$ 0.2 at 25°C. After sterilization, pour the melted medium into sterilized petri plates (approximately 20 to 30 ml per plate) and let it solidify before use. Prepared medium is clear to slightly hazy and pale yellow. Prepared plates can be stored in the refrigerator for up to 4 weeks before use. Allow the medium to come to room temperature before inoculation.

PEA agar with 5% sheep blood typical composition (g/liter) (1, 2, 12)

Pancreatic digest of casein	15 g
Papaic digest of soybean meal	5 g
Sodium chloride	5 g
β-Phenylethyl alcohol	2.5 g
Sterile defibrinated sheep blood	50 ml
Agar	15 g
Distilled water	1.0 liter

Suspend all ingredients except sheep blood in 1 liter of distilled water and mix thoroughly. Heat with frequent agitation and boil for 1 minute to dissolve completely. Autoclave the medium at 121°C for 15 minutes at 15 psi. Final pH of the medium should be 7.3 ± 0.2 at 25°C. Cool to 45°C and add 5% sterile defibrinated blood and mix well. Quickly pour the melted medium into sterilized petri plates (approximately 20 to 30 ml per plate) and let it solidify before use. Prepared medium appears firm, opaque, and red in color. Prepared plates could be stored in the refrigerator up to 1 week before use. Allow the medium to come to room temperature before inoculation.

PEA agar medium is also commercially available as premixed powder from biological supply companies. The manufacturer's instructions should be followed to prepare the plates. This media can also be purchased as premade agar plates from biological supply companies.

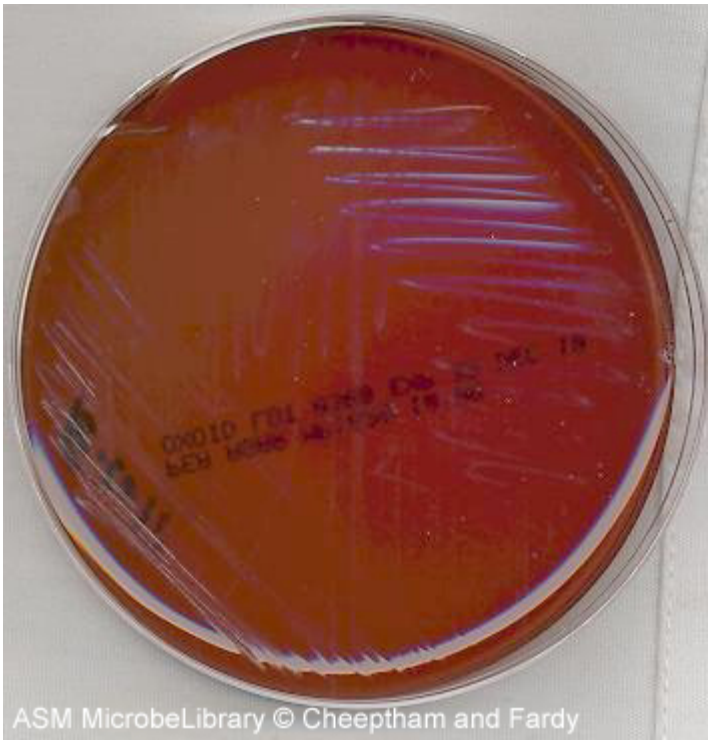
PROTOCOL (1, 2, 12)

Inoculation. Aseptically transfer potentially mixed cultures onto the surface of the agar using a four-way streaking technique. Depending on the objectives of the study, either a confluent growth or a four-way streaking technique can be used.

Incubation. Incubate plates for 24 to 48 hours at 35°C ± 2°C in an appropriate atmosphere. In some cases, a longer incubation, up to 1 week, may be needed. PEA blood agar plates can be incubated under aerobic, anaerobic, and 5% CO_2 atmosphere based on the type of microorganisms being studied. Incubation in high CO_2 atmosphere allows the detection of bacteria which require an increased CO_2 concentration and also results in better growth of almost all of the other pathogens (5).

Interpretation of results. After proper incubation, growth of isolated colonies or a group of colonies may be observed. Gram-positive bacteria demonstrate good growth (Fig. 1c and 2c) while most gram-negative bacteria do not grow or are partially inhibited (Fig. 1b and 2b).

a



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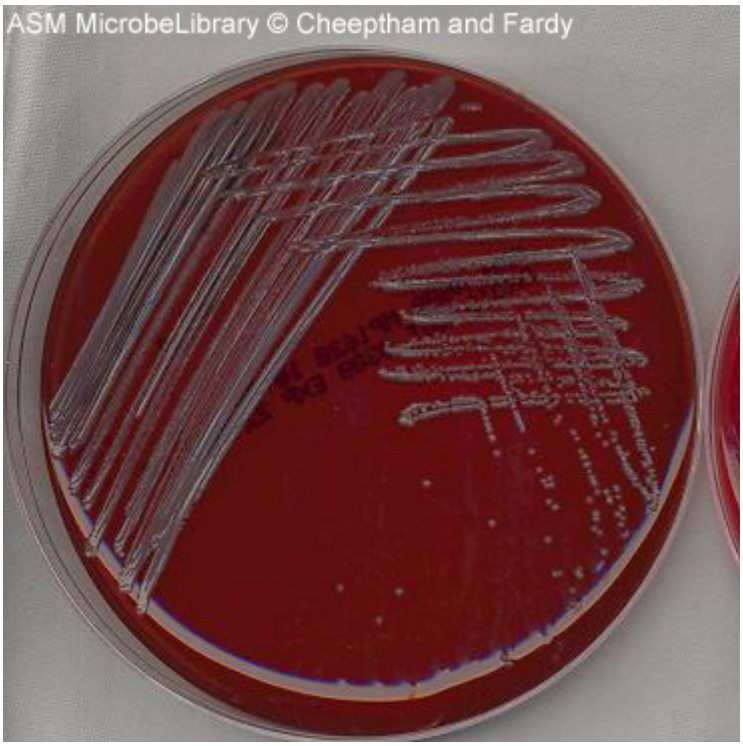

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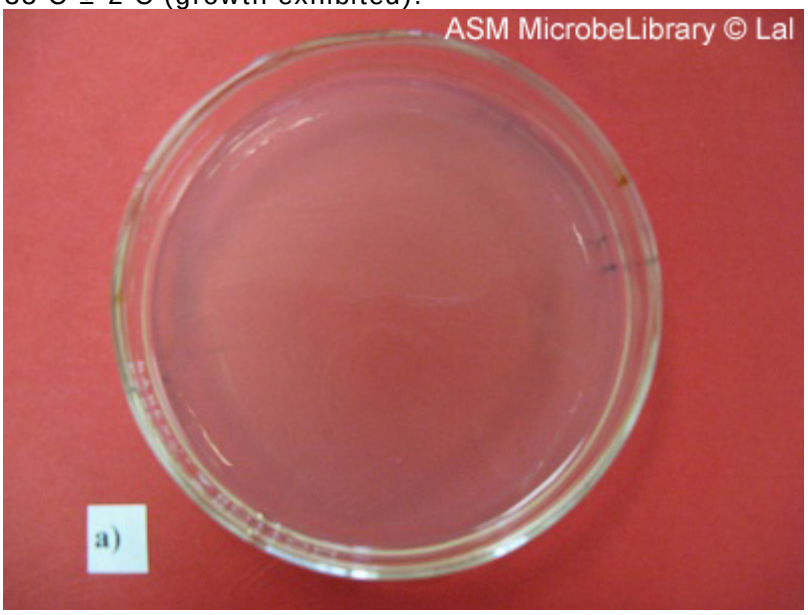
FIG. 1. PEA agar plates with 5% sheep blood: (a) an uninoculated PEA agar plate with 5% sheep blood, (b) a PEA agar plate with 5% sheep blood inoculated with *Escherichia coli*, a gram-negative bacteria, incubated under 5% CO_2 for 48 hr at 35°C ± 2°C (growth inhibited), and (c) a PEA agar plate with 5% sheep blood inoculated with*Staphylococcus*

aureus, a gram-positive bacteria, incubated under 5% CO_2 for 48 hr at 35°C ± 2°C (growth exhibited).



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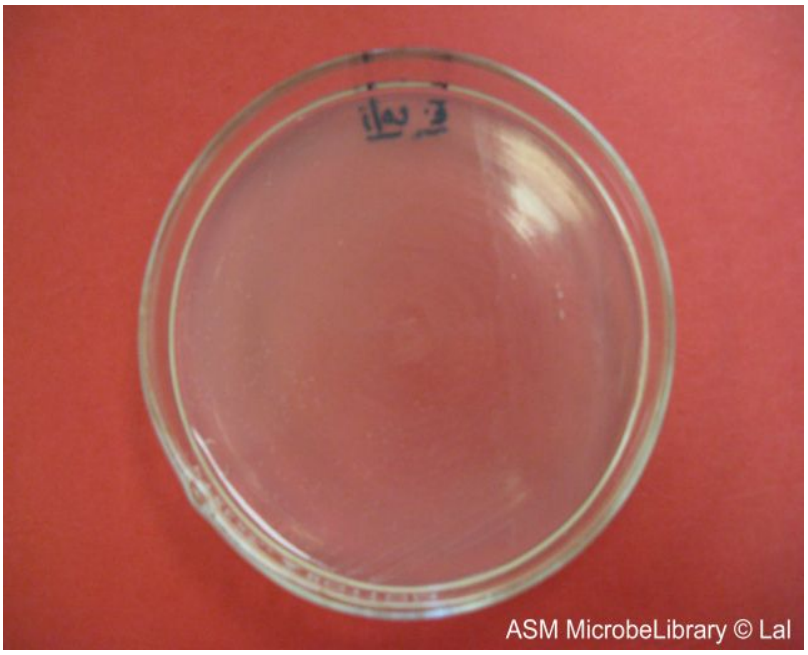

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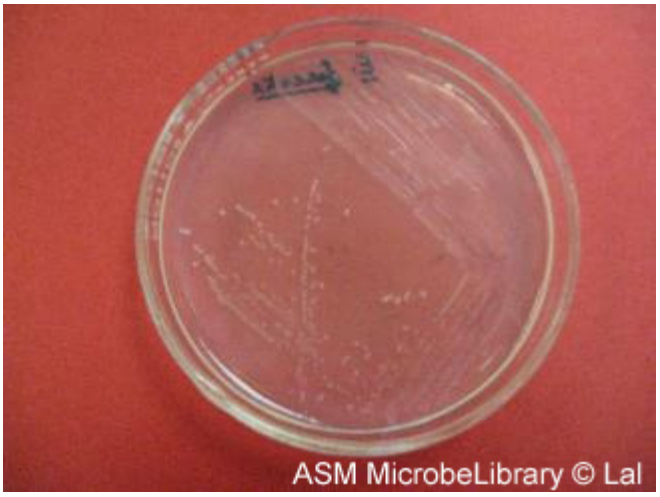

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FIG. 2. PEA agar plates: (a) an uninoculated PEA agar plate, (b) a PEA agar plate inoculated with *Escherichia coli,* a gram-negative bacteria, incubated under aerobic conditions for 48 hr at 35°C ± 2°C (growth inhibited), and (c) a PEA agar plate inoculated with *Enterococcus faecalis,* a gram-positive bacterium, incubated under aerobic conditions for 48 hr at 35°C ± 2°C (growth exhibited).

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

1. PEA agar with 5% sheep blood should not be used for determination of hemolytic reactions as irregular patterns may be observed (1, 2, 12). Organisms should be subcultured onto tryptic soy agar with 5% sheep blood to examine hemolysis.
2. Some gram-positive cocci may be slightly inhibited by PEA and many require incubation up to 48 hours for sufficient growth to be visible (2).
3. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on PEA agar medium.
4. *Pesudomonas aeruginosa* (a gram-negative bacteria) is not inhibited on this medium (2, 5).
5. In order to control for the viability of the organisms used, a control nutrient agar or other nonselective medium should be used in parallel.
6. It is important to remember that this medium inhibits the growth of gram-negative bacteria. Tiny observable colonies on PEA agar may be gram-negative microorganisms and are often confined to first quadrant on a streak plate.

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