SAMUEL ADEGBOYEGA UNIVERSITY

College of Basic and Applied Sciences

Department of Biological Sciences

MCB 223 (Microbiological Techniques)

Culture Media and Their Preparations

By

ENERIJIOFI, K. E., *Ph. D*

Outline

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Definitions of Terms

- **Culture media:** These are food substances required by microorganisms for growth.
- **Agar:** This is a purified carbohydrate obtained from marine seaweed which is widely used as a solidifying agent.
- **Fastidious Bacteria:** These are bacteria that need an extra nutrient for growth. They can be said to be nutritionally exacting.

History of culture media

- Louis Pasteur used simple broths made up of urine or meat extracts to grow bacteria.
- Gelatin was used as a solidifying agent but it had two issues;
- 1. It existed as liquid at normal incubating temperatures (35-37^{o}C)

History of culture media cont'd

- 2. It was digested by certain bacteria.
- Robert Koch realized the importance of solid media and used potato pieces to grow bacteria.
- Thereafter, Fannie Eilshemius came up with the idea that agar could be used to solidify culture media.

Composition of culture media

Most often, a culture medium contains

- Water;
- a source of carbon and energy;
- source of nitrogen;
- trace elements;
- some growth factors;
- Agar;

Composition of culture media cont'd

- Peptone;
- casein hydrolysate;
- meat extract;
- yeast extract and malt extract.

Composition of Tryptic Soya Broth

Component	g/L medium
Tryptone	17.0
Soytone	3.0
Nacl	5.0
K_2HPO_4	2.5
Glucose	2.5
Total	30.0

Preparation of Culture Media

- Rehydrate tablets or powder according to manufacturer's instructions.
- Ensure ingredients are completely dissolved, use heat if necessary.
- Adjust the pH of the medium using pH indicators like phenol red, neutral red, bromothymol blue and bromocresol purple etc.
- Sterilize by autoclaving.

Preparation of Culture Media cont'd

- However, certain media that contain heat labile components like glucose, antibiotics, urea, serum and blood are not autoclaved.
- These components are filtered and added separately after the medium is autoclaved.
- The sterilize culture media is allowed to set at normal temperature.

Pouring a Plate / Petri Dish

The procedures for pouring a plate / petri dish are outlined below:

- Collect a bottle of sterile molten agar from the water bath.
- Hold the bottle in the left hand and remove the lid with the little finger of the right hand.
- Flame the neck of the bottle
- Lift the lid of the Petri dish slightly with the right hand and pour the sterile molten agar into the Petri dish and replace the lid.

Pouring a Plate / Petri Dish cont'd

- Flame the neck of the bottle and replace the lid.
- Gently rotate the dish to ensure that the medium covers the plate evenly.
- Allow the plate to solidify.
- Seal and incubate the plate in an inverted position.
- **NOTE: The base of the plate must be covered, agar must not touch the lid of the plate and the surface must be smooth with no bubbles.**

Storage of Culture Media

Culture media can be stored as stated below

- Store stocks of prepared media at room temperature away from direct sunlight.
- Sterile agar plates can be pre-poured and stored in well-sealed plastic bags.

Classification of Culture Media

- Bacterial culture media can be classified in three ways namely

- Based on consistency e. g Liquid media, Solid media, Semi - solid media and Biphasic media ;

- Based on nutritional component e. g simple media, complex media synthetic or defined media

- Based on functional use or application e. g basal media, enriched media, selective media, differential media, transport media and anaerobic media.

Based on consistency

- **Liquid media:** They are available for use in test tubes, bottles or flasks.
- Liquid media are sometimes referred as broths e. g nutrient broth.
- In liquid medium, bacteria grow uniformly producing general turbidity.
- Liquid media tend to be used when a large number of bacteria have to be grown.
- The properties of bacteria are not visible in liquid media and
- The presence of more than one type of bacteria cannot be detected

Based on consistency cont'd

- **Solid media:** It melts at 95^{o}C and solidifies (gels) at 45^{o}C.
- Any liquid medium can be rendered by the addition of certain solidifying agents called agar
- Agar is composed of two long-chain polysaccharides (70% agarose and 30% agarapectin).
- E. g Nutrient agar, Blood agar, MacConkey agar e.t.c

Based on consistency cont'd

- **Semi-solid agar:** They are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains.
- Reducing the amount of agar to 0.2 - 0.5% renders a medium semi solid.
- Examples are Stuart's and Amies media, Hugh & Leifson's oxidation fermentation test medium and mannitol motility medium.

Based on consistency cont'd

- **Biphasic media:** This is a culture system that comprises of both liquid and solid medium in the same bottle.

- The inoculum is added to the liquid medium and when subcultures are to be made, the bottle is simply tilted to allow the liquid to flow over the solid medium.

- E. g Loeffler's serum slope, Lowenstein Jensen medium and Dorset egg medium

Based on Nutritional Component

- **Simple media:** These are media that can support most non-fastidious bacteria. E. g include peptone water and nutrient agar.

- **b) Complex media:** They are used for growing fastidious bacteria. E. g Blood agar.

- **c) Synthetic or defined media:** In this, their composition is well known. E. g include Davis & Mingioli medium, Nutrient agar, Eosine Methylene Blue agar.

- Synthetic agar are specially prepared media for research purposes

Based on functional use or application

- **Basal media:** They are basically simple media that supports most non-fastidious bacteria. E. g Peptone water, nutrient broth and nutrient agar.
- **Enriched media:** These are media where extra nutrients have been added and are used to grow fastidious bacteria.
- E. g include Blood agar, chocolate agar and Loeffler's serum slope e. t .c
- **Selective media:** They are designed to help in recovering pathogen from a mixture of bacteria.
- Any media can be made selective by adding some inhibitory agents like antibiotics, dyes. E. g of selective agar include Wilson & Blair's medium and TCBS agar.

Based on functional use or application cont'd

- **Transport media:** They are to transfer clinical specimens. Such media prevent desiccation of the specimen and maintain the pathogen to commensal ratio.
- E. g are Stuart's & Amie's, Cary Blair medium and Venkatraman Ramakrishnan medium and Sach's buffered glycerol saline.
- **g) Anaerobic media:** They are special media for growing anaerobic bacteria because they need low oxygen content, reduced redox potential and extra nutrients. E. g Thioglycollate medium
- Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K.

Reasons for culturing Microorganisms in Microbiology

- Its use in diagnosing infectious diseases.
- Indication of its role in the disease process.
- Initial step in studying its morphology and identification.
- To obtain antigens from developing serological assays or vaccines.
- Genetic studies and manipulations of the cells in vitro.
- Provide a reliable way of estimating microbial numbers (viable count).
- Convenient way of separating bacteria in mixtures.

Conclusion

- Culture media and their preparations is very apt because it enable microbiologists to study diverse microorganisms from different sources for the benefit of man and improvement in the society.

References

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