SELECTIVE MEDIA FOR ISOLATING YEASTS FROM FECES

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RESEARCH ARTICLE

Abstract

Understanding the nutritional requirements of bacteria is highly important in bacteriology, as it forms the foundation for the preparation of culture media designed for isolating and cultivating microorganisms in the laboratory, whether for diagnostic or productive purposes. Culture media are mixtures of nutritive substrates used to sustain the viability, growth, and multiplication of microorganisms under artificial conditions. The use of culture media allows for the isolation of microorganisms in pure culture, thereby facilitating their identification. They are also employed for testing the sensitivity of microorganisms to antimicrobial agents, enabling the selection of targeted medication. In the pharmaceutical industry, they are utilized for the production of specific preparations and for monitoring the microbial load of surfaces, air, and food.

Keywords: culture media, microorganism, multiplication

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INTRODUCTION

For diagnostic or productive purposes, culture media are prepared from a series of biologically derived substrates, more or less chemically well-defined, which are found in almost all culture media.

In research, when studying the metabolic performance of bacteria, media with a very precisely defined composition are required. These media are prepared from each substance individually and are called synthetic media.

The biological substrates commonly encountered in most media include peptones, meat extract, yeast extract, to which certain substances such as sodium chloride, mono- and/or polysaccharides, and vitamins are added.

Peptones are mixtures of substances obtained through enzymatic or acid hydrolysis of animal-derived proteins. They do not have a very well-defined chemical composition but, due to their content of peptides and amino acids, they serve as a universal source of nitrogen for all cultivable bacteria. They are practically used in the preparation of all culture media.

Meat extract is obtained by dehydrating beef broth. It contains significant quantities of creatine, xanthine, hypoxanthine, uric acid, adenylic acid, glycocoll, urea, glutamine as a nitrogen source, as well as glycogen, hexose phosphates, lactic acid as a carbon source, etc. It can be prepared in any laboratory.

east extract is obtained through controlled cultivation of yeasts and contains numerous vitamins, especially those from the B group.

Sodium chloride is added to all standard media at a concentration of 0.9%. For the cultivation of halophilic bacteria (which grow at high osmolarity), the concentration can be increased up to 10%.

Mono-or polysaccharides and certain alcohols enrich the media as they serve as easily accessible carbon sources for many bacteria. Sometimes, colorimetric pH indicators and selective agents are added to the media. In the preparation of solid media, solidifying agents of particular importance are agar-agar and gelatin. Gelatin is used relatively rarely because, on the one hand, some bacteria hydrolyze it, and on the other hand, it liquefies at 37°C, the temperature at which most medically relevant bacteria grow.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the medical analysis laboratory, S.C. Diaser, Oradea. To conduct the study, I also accessed the archive, recorded in the laboratory's computer program in S.C. Diaser, Oradea, as well as the computerized database of the unit.

Materials required for the examination:

- A collection container (a collection container with a spoon) with transport medium
- Wooden spatulas
- Latex gloves

For a stool culture, a fecal sample of 5-10g should be collected and placed in the collection container with a transport medium. If the stool is liquid, 5ml should be collected. It is recommended to choose a liquid, mucous, or bloody portion, if available. Do not collect quantities greater than 10g, as they may reduce the chances of isolating pathogenic bacteria.

solation of aerobic bacteria. • The sample is inoculated onto two culture media, one weakly selective (MacConkey) and one moderately selective (Hektoen), and incubated for 24 hours at 35-37°C. The cultures are observed at 24 and 48 hours for the appearance of characteristic colonies. For the genus Vibrio, the recommended selective medium is BSA (bile salts agar), and for yeasts, Sabouraud medium with Chloramphenicol is used. • To increase the chances of isolation, the sample is subcultured on enrichment media that promote the multiplication of pathogens (selenite sodium acid broth for Salmonella spp., alkaline peptone water or broth with taurocholate and peptone at pH 8.0-9.0 for Vibrio, from which after incubation, smears and cultures can be performed from the upper part of the medium). Incubation is done for 24 hours at 35-37°C, followed by transfers to culture media.

RESULTS AND DISCUSSIONS

Microscopic appearance is crucial for culturing stool samples for the isolation and quantification of yeasts. Yeasts are always intensely Gram-positive and, depending on their taxonomic group, can assume various forms, such as spherical or oval yeast cells arranged in clusters or frequently branched moniliform chains. Their arrangement and morphology can suggest specific species, which is why the microscopic examination of the specimen and subsequent isolation is essential in mycological analysis.

The isolation of yeasts is achieved through cultivation on agar-based media containing inhibitors for bacteria. The preliminary steps leading to cultivation are the same as those for bacteriological investigation, with the important note that the collection is always performed from spontaneously emitted stool.

Table 1

Selective Media for the Isolat
ion of Yeasts from Stool

Medium	Abbreviation.	Nutritional compositio	Selective composition	Incubation at 25°C or 35°C
Sabouraud	Sabouraud CCG	Glucose Bactopeptonă NaCl Agar	Cloramfenicol Cycloheximidă Gentamicină	Selective for C. albicans
Sabourand with dextroză Cloramfenicol	Sabouraud DC	Glucose Neopeptonă Agar	Cloramfenicol	Selective for fungi

Table 1 presents two recommended selective media for isolating yeasts from samples with a rich bacterial flora. Both are variations of Sabouraud medium supplemented with mixtures of antibiotics inhibitory to aerobic bacteria from fecal matter.
To incriminate a mycotic etiology in a diarrheal syndrome, a quantitative examination of yeasts is necessary, including determining the number of yeast colony-forming units (CFUs) per gram or milliliter of stool and identifying the specific yeasts.
From the initial fecal suspension, in a saline solution, dilutions of 10^(-2) and 10^(-3) (or 10^(-3) and 10^(-4) of the product, respectively) are prepared. From these dilutions, 0.2 ml each is inoculated onto a Sabouraud DC plate by flooding. Simultaneously, dispersions are made from the initial suspension using a loop on Sabouraud CCG.

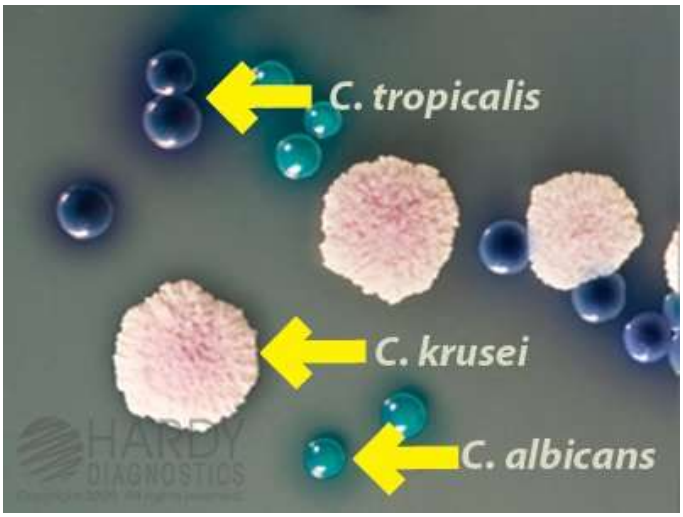

Figure 1. **Typical Colonies for C. Albicans, C. Krusei, C. Tropicalis**

Preliminary identification is based on colony characteristics and microscopic features (shape, dimensions, presence of a capsule).

Candida albicans plays a major role in the mycotic etiology of diarrhea syndrome. Its presence, especially in immunodeficient individuals, represents one of the recurring infections frequently seen in AIDS. The culturability aspects of C. albicans are distinctive on Sabouraud CCG agar, being the only yeast that grows with such a high degree of selectivity.

The study on "Immobilization of filamentous fungi: A new frontier in organic acid production" highlights some primary or secondary fungal metabolites, as well as enzymes and other biotransformation products of fungal origin, which play a strategic role in various technologies, particularly in food processing. Organic acids, such as citric acid, are of significant interest. Citric acid is already produced at over 3 tons per year, and the market demand for other acids like gluconic, lactic, and malic acid is continuously growing. This review mainly focuses on the latest advancements in the production of organic acids from immobilized fungal cell systems. The production of fumaric acid by immobilized Rhizopus arrhizus is reported as a case study. In fact, the fermentative production of this acid, which was long abandoned for economic reasons and replaced with direct chemical synthesis, could greatly benefit from the utilization of immobilized cell-based technology.

CONCLUSIONS

Sabouraud CCG medium is highly selective, allowing the growth of only C. albicans.

The Sabouraud DC variant is less inhibitory, permitting the growth of other yeasts as well, making it more versatile in its applications.

Enrichment in liquid media with suitable inhibitors is important for isolating C. Jejuni from food samples, while direct plating on appropriate selective media is preferred for isolating it from fecal specimens.

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