Sabouraud Agar for Fungal Growth Protocols

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Author

- Janelle Hare

Information

History

Sabouraud (pronounced sah-bū-rō′) agar medium was developed by the French dermatologistRaymond J. A. Sabouraud in the late 1800's to support the growth of fungi that cause infection of the skin, hair, or nails, collectively referred to as dermatophytes (5,6). Sabouraud's medical investigations focused on bacteria and fungi that cause skin lesions, and he developed many agars and techniques to culture pathogenic moulds and yeasts, such as dermatophytes and *Malassezia*. He particularly desired that all mycologists detail their exact media formulations, temperatures and times of incubation of specimens, in order to standardize the field's observations and thus reduce differences in appearance as a possible source of error in identification (4).

Purpose

Historically, Sabouraud agar was developed to support the studies of dermatophytes, which require long incubation periods (weeks). There were two driving forces behind Sabouraud's development of this medium: the need to avoid bacterial contamination while culturing dermatophytes and other fungi, and the need to provide a medium that would yield reliable results for fungal identification across laboratories. Sabouraud agar is a selective medium that is formulated to allow growth of fungi and inhibit the growth of bacteria. The available means of inhibiting bacterial growth in Sabouraud's pre-antibiotic era was an acidic medium (pH 5.6). However, the addition of antibiotics to the acidic medium to inhibit bacteria (and sometimes saprophytic fungi, depending on the particular antibiotics used) is common in currently used versions. Glucose is present at the high level of 4% in Sabouraud's formulation to assist in vigorous fermentation and subsequent acid production by any bacteria present. High acid concentrations eventually serve to inhibit all bacterial growth.

Theory

The medium is complex but contains few ingredients. Peptones, as soluble protein digests, are sources of nitrogenous growth factors that can vary significantly according to protein source. Sabouraud's original formulation contained a peptone termed "Granulée de Chassaing," which is no longer available (4). (This may be why the standard name for this medium is "Sabouraud agar, modified.") Variations in pigmentation and

sporulation can be consistently observed if one uses**Sabouraud** medium prepared with consistent ingredients, because morphology can vary slightly based on the peptones used. Both Difco and BBL Sabouraud agars use pancreatic digests of casein as their peptone source. Although Sabouraud originally used the sugar maltose as an energy source, glucose (or dextrose, as it used to be called), is currently used, and agar serves to solidify the medium.

RECIPES AND PROTOCOLS

Sabouraud agar can be purchased from a variety of commercial sources, either as the original recipe (Sabouraud agar, modified), or in a slightly altered version termed "Sabouraud agar, Emmons." The neutral pH of the Emmons modification seems to enhance the growth of some pathogenic fungi, such as dermatophytes.

Per liter of medium:
Peptone, 10 g
Glucose, 40 g
Agar, 15 g

1. Combine all ingredients in ~900 ml of deioinized water.
2. Adjust to pH 5.6 with hydrochloric acid and adjust final volume to 1 liter.
3. Autoclave 20 minutes at 121°C, 15 lb/in^2.
4. Cool to ~45 to 50°C and pour into petri dishes or tubes for slants.

Emmons modification of Sabouraud agar (1)
Per liter of medium:
Neo-peptone, 10 g
Glucose, 20 g
Agar, 20 g

1. Follow steps 1 through 4, above, except adjust the pH to the range of 6.8 to 7.0 with hydrochloric acid before autoclaving, cooling, and pouring.

Either Sabouraud agar or its Emmons version can be made more selective by adding antibiotics. Commonly used are gentamicin, which inhibits gram-negative bacteria, and/or chloramphenicol, which inhibits a wide range of gram-positives and gram-negatives, and cycloheximide, which inhibits primarily saprophytic fungi but not dermatophytes or yeasts (3). Chloramphenicol and gentamicin are used at 50 mg/liter (dissolved in 10 ml of 95% ethanol before adding to molten media) and cycloheximide at 0.5 g/liter (dissolved in 2 ml of acetone first) (2). Antibiotics should only be added after media has been autoclaved and then cooled to ~45 to 50°C. Keep all plates at 4°C until they are used, regardless of whether they contain antibiotics.

Sabouraud agar plates can be inoculated by streaking (see the Streak Plate Protocol for an explanation of this method), as with standard bacteriological media, or by exposing the medium to ambient air. Typically, molds are incubated at room temperature (22 to 25°C)

and yeasts are incubated at 28 to 30°C or 37°C if suspected of being dimorphic fungi. Incubation times will vary, from approximately 2 days for the growth of yeast colonies such as *Malasezzia*, to 2 to 4 weeks for growth of dermatophytes or dimorphic fungi such as *Histoplasma capsulatum*. Indeed, the incubation time required to acquire fungal growth is one diagnostic indicator used to identify or confirm fungal species.

SAFETY

Some *Trichophyton* species require additional growth factors, such as thiamine and inositol (*T. verrucosum*) or nicotinic acid (*T. equinum*), so may not grow well, if at all, on Sabouraud agar.

Sabouraud agar is sometimes referred to as Sabouraud's dextrose agar or Sabouraud's agar.

Mold morphology should be observed on both the top (obverse) and bottom (reverse) surfaces, as differences can be seen on each surface. Some safe fungi for undergraduate students include the molds *Penicillium camemberti* and*P. roqueforti* (used in making cheeses), *Rhizopus stolonifor* (used in making tempeh),*Aspergillus* species except *A. fumigatus* and *A. flavus*, and the yeasts *Saccharomyces cerevisiae, Rhodotorula rubrum,* and *Neurospora crassa*. Fungi often produce spores that are easily dispersed into the laboratory upon opening of plates.

Plates should be wrapped in parafilm to maintain them securely closed and should be incubated with the lid on the top (as opposed to the typical practice of inverting plates for incubation) to avoid spreading spores if the plates are opened. Culture plates should be opened only within a biosafety cabinet to avoid contamination of laboratory spaces with fungal spores, possible infection of individuals by pathogenic fungi, or induction of allergic responses.

The Curriculum Resources Committee recognizes that isolated organisms can be a powerful learning tool as well as a potential biological hazard. We strongly recommend that:

- Environmental enrichment laboratories should only be performed in classes in which students have been trained to work at biosafety level 2.
  Direct environmental samples (e.g., soil, water) which are known to contain infectious organisms should be handled according to the biosafety level of that infectious agent.
- Cultures of enriched microorganisms, derived from environmental samples, should be handled using biosafety level 2 precautions.
- Mixed, enriched, or pure cultures of microorganisms from environmental samples with a significant probability of containing infectious agents should be manipulated in a biosafety cabinet if available.
- Where possible, media used for the enrichment of environmental isolates should contain an appropriate antifungal agent.
- Instructors should be aware if they are teaching in regions with endemic fungi capable of causing systemic infections and should avoid

environmental isolations.

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.

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