Mannitol Salt Agar Plates Protocols

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Information

History

Early work by Gordon indicated that the fermentation of mannitol could be used as a means of differentiating pathogenic staphylococci from nonpathogenic staphylococci (12). Although his work was confirmed and expanded upon by several investigators (Dudgeon, 1908, 10), mannitol fermentation was not used routinely as an identification tool. Winslow and his coworkers set out to perform a "more exhaustive series of quantitative tests" on the differentiation of staphylococci and concluded that certain sugars, including mannitol, "are attacked by the staphylococci so rarely as to be of no serious diagnostic value" (18).

In addition to the problems encountered with the differentiation of staphylococci, the isolation of staphylococci also proved to be problematic. The solid media used at the time not only supported the growth of both pathogenic and nonpathogenic staphylococci but other contaminants as well. As such, methods used for the isolation of probable pathogenic staphylococci from suspected sources usually involved a series of isolation steps and tests including determination of hemolysis, coagulation tests, pigment production, and agglutination tests (2, 6, 7, 16).

At the Clinical Research Laboratory in New York, George Chapman and his coworkers developed a series of media for the isolation of possible pathogenic staphylococci in the 1930s and 1940s. These media included crystal violet agar (4) and bromthymol-blue lactose agar (6). During this time, Chapman's lab began to reexamine mannitol fermentation and confirmed the very early work of Gordon (7). Their work showed that a combination of bromthymol-blue lactose agar and phenol-red mannitol agar was very reliable for the isolation and differentiation of possible pathogenic staphylococci (5, 8).

Finally, in 1942, Koch discovered that the presence of 7.5% sodium chloride in media inhibited the growth of most organisms except staphylococci (14). Chapman utilized this information to modify the formula of the phenol-red mannitol agar and mannitol salt agar was the result (3).

Purpose

Mannitol salt agar (MSA) is both a selective and differential medium used

in the isolation of staphylococci. It contains 7.5% sodium chloride and thus selects for those bacteria which can tolerate high salt concentrations. MSA also distinguishes bacteria based on the ability to ferment the sugar mannitol, the only carbohydrate in the medium.

Theory

Staphylococci can withstand the osmotic pressure created by 7.5% NaCl, while this concentration will inhibit the growth of most other gram-positive and gram-negative bacteria (14). Additionally, MSA contains mannitol and uses phenol red as a pH indicator (pK = 7.8) in the medium. At pH levels below 6.9, the medium is a yellow color. In the neutral pH ranges (6.9 to 8.4) the color is red; while above pH 8.4, the color of phenol red is pink (11). When mannitol is fermented by a bacterium, acid is produced, which lowers the pH and results in the formation of a yellow area surrounding an isolated colony on MSA. A nonfermenting bacterium that withstands the high salt concentration would display a red to pink area due to peptone breakdown (15).

In clinical samples, mannitol positive isolates are suggestive of *Staphylococcus aureus* and should be tested further.

RECIPE

Beef extract	1.0 g
Peptone (Difco) or Polypeptone (BBL)	10.0 g
NaCl	75.0 g
Mannitol	10.0 g
Phenol red	0.025 g
Agar	15.0 g
Distilled water	1,000 ml

Adjust pH to 7.4. Boil to dissolve agar. Sterilize at 121^{o}C for 15 minutes. Cool to 55^{o}C and pour into petri dishes.

Mannitol salt agar is also commercially available in a premixed form.

PROTOCOL

Streak a plate of mannitol salt agar with appropriate culture using the quadrant streak plate method to obtain isolated colonies. Well-isolated colonies will provide the best results in the biochemical differentiation bacteria using MSA.

FIG. 1. Tryptic soy agar (TSA) inoculated with (A) *Staphylococcus aureus,* (B) *Staphylococcusepidermidis,* and (C) *Escherichia coli* demonstrating growth of all three organisms. TSA is a general purpose medium that will allow for the growth of all three organisms.

FIG.
2. (A) *Staphylococcus aureus,* (B) *Staphylococcus epidermidis,* and (C) *E scherichia coli* streaked on a mannitol salt agar plate.
The mannitol fermenting colony (yellow) is *S. aureus*, while themannitol nonfermenting colony (pink) is *S. epidermidis*. The growth of *E. coli* was inhibited by the high salt concentration.

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

- Media color change demonstrates mannitol fermentation, NOT colony color. This is particularly important as many micrococci are pigmented.
- Inoculated plates that are kept refrigerated may exhibit color loss over time. Some instructors have found that reincubation may bring back some color. Others have indicated that pouring plates thicker lessens the color loss.

Please contact the project manager with additional feedback.

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