

Mannitol Salt Agar

Theory

Mannitol salt agar (MSA) contains the carbohydrate mannitol, 7.5% sodium chloride (NaCl), and the pH indicator phenol red. Phenol red is yellow below pH 6.8, red at pH 7.4 to 8.4, and pink at pH 8.4 and above. Mannitol provides the substrate for fermentation and makes the medium differential. Sodium chloride makes the medium selective because its concentration is high enough to dehydrate and kill most bacteria.

Staphylococci thrive on the medium, largely because of their adaptation to salty habitats such as human skin. Phenol red indicates whether fermentation with an acid end-product has taken place by changing color as the pH changes. (See Section 5, p. 268, "A Word About Biochemical Tests and Acid-Base Reactions.")

Most staphylococci are able to grow on MSA, but do not ferment mannitol, so their growth appears pink or red and the medium remains unchanged. *Staphylococcus aureus* ferments mannitol, which produces acids and lowers the pH of the medium (Fig. 4-3). The result is formation of bright yellow colonies usually surrounded by a yellow halo (Fig. 4.4). For more information on fermentations, refer to Exercise 5-2 and Appendix A.

Application

Mannitol salt agar is used for isolation and differentiation of *Staphylococcus aureus* from other *Staphylococcus* species. It is not used to determine the ability of an isolate to ferment mannitol. A fermentation broth, such as phenol red mannitol, would be used instead (see Exercise 5-2).



4

4.4 Mannitol Salt Agar ■ This medium is selective for members of the genus *Staphylococcus* due to their salt tolerance. It further allows differentiation between *Staphylococcus* species based on the ability to ferment mannitol to acid end products (top) and those that do not (bottom). MSA would normally be streaked for isolation, but you will spot inoculate pure cultures for the sole purpose of seeing what the different results look like.

In This Exercise

Today you will spot inoculate one MSA plate and one nutrient agar (NA) plate with three test organisms. (In a clinical application, MSA plates would be streaked for isolation with a patient's sample.) The NA will serve as a comparison for growth quality on the MSA plate.



4.3 Mannitol Fermentation with Acid End-Products ■ There are two important points to note here: one, that the fermentation provides a mechanism for oxidizing NADH back to NAD⁺ so it can be used again in glycolysis and two, that the end product of the fermentation is an acid—the acid detected by the phenol red pH indicator in MSA. For simplicity, only the relevant parts of glycolysis are shown. See Appendix A, Figure A.1 for details.

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Table of Contents Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers

1. Understanding the eBook Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - The Rise of Digital Reading Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Personalized Recommendations
 - Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers User Reviews and Ratings
 - Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers and Bestseller Lists
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 - Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers Enhanced eBook Features
- 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Highlighting and Note-Taking Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Interactive Elements Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
- 8. Staying Engaged with Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
- 9. Balancing eBooks and Physical Books Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Setting Reading Goals Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Microbiology Laboratory Theory And Applications Third Edition Data Sheet Answers
 - Fact-Checking eBook Content of Microbiology Laboratory Theory And Applications Third Edition Data Sheet

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- 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
- 14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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