

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).



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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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