Capsule Stain Protocols

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Information

History

Since the 1900s various methods have been devised to observe bacterial capsules (7). One very simple approach is mixing cells in a preparation of India ink. The large particles of ink will not penetrate the tight layers of the capsule or stain the bacterium. The particles of the ink will however

capsule of Bacillus anthracis is composed of polymers of amino acids (10).

In the case of human pathogens, a large number of different capsule serotypes have been identified. For example, over 80 different capsular polysaccharides or K antigens have been described for Escherichia coli (13, 15). For Streptococcus pneumonieae, over 90 different serotypes exist defined by unique antigenic components of capsular polysaccharides. The presence of the capsule is required for virulence in Streptococcus pneumonieae disease, and capsule antigenic variation provides significant challenge to vaccine efforts (14). Beta-hemolytic streptococci have been classified serologically on the basis of Lancefield group antigens found in the capsule.

Capsules are considered protective structures. Various functions have been attributed to capsules including protection from desiccation (11, 13) and adherence to surfaces and other bacteria contributing to biofilm formation (13). Capsules also often play a role in pathogenicity (3) acting as virulence factors to protect cells from phagocytosis and/or complement-mediated killing. Important plant pathogens such as strains of Pseudomonas, Rhizobium, and Erwinia require capsules for pathogenicity (13).

The size and constituency of the capsule varies with species and strains. Biosynthesis and assembly of capsules is a complex process (12, 15). The synthesis of the capsular material depends upon the environment, and for specific strains of bacteria, capsules are not required at all times. Organisms lacking capsules grow well at least under laboratory conditions, but it is important to note that these layers are essential for survival in certain natural environments and in some cases responsible for virulence of bacterial pathogens (e.g., Streptococcus pneumonieae). In the laboratory, it is common that special media such as milk broth or litmus milk broth are used to support the growth of encapsulated strains of bacteria.

Capsular polysaccharides are highly hydrated molecules containing over 95% water (13). As such, capsules are best demonstrated in preparations without heat fixation as heat will cause distortion and shrinking of the capsule.

Capsules are characterized by poor staining with standard dyes. Capsule staining methods thus depend upon revealing the presence of the capsule indirectly. Often capsule staining methods are accomplished using a combination of the following: (i) a basic dye that interacts with the negative ions of the bacterial cell, (ii) a mordant that causes the precipitation of the capsular material, e.g., metal ions, alcohol, and acetic acid (9), and (iii) an acidic stain used to color the background.

At the completion of the preparation, the capsule is revealed as a clear halo between the colored background and the stained cell. In some capsule staining preparations, cells are exposed to antibody against capsular antigens to enlarge the capsule for easier visualization (9).

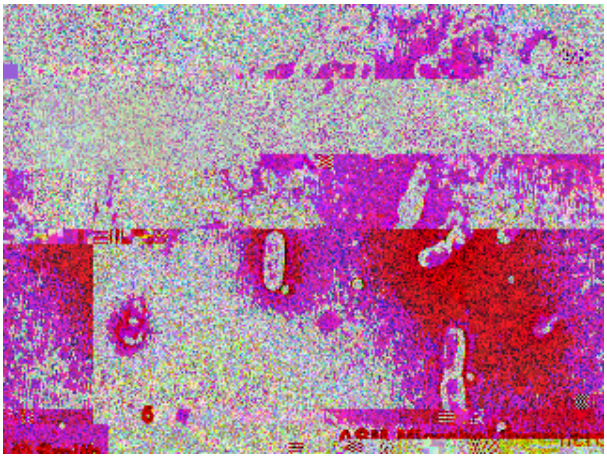

FIG. 4. Encapsulated Bacillus sp. stained using Maneval's capsule staining method. Note that the capsule is seen as a clear halo around the rod-shaped bacterium.

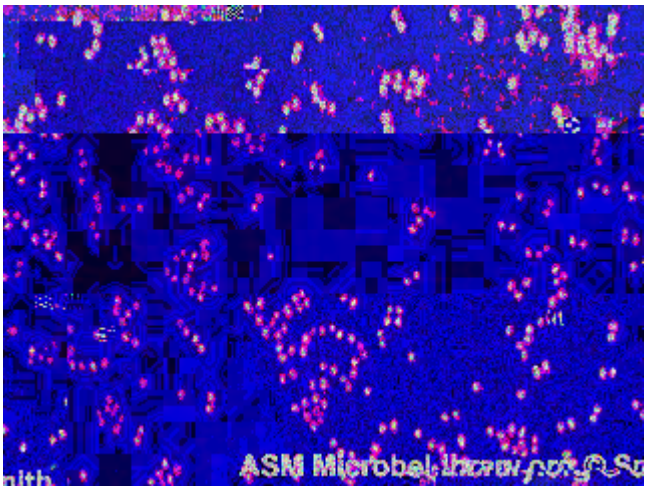

FIG. 5. Staphylococcus epidermidis stained using Maneval's capsule staining method. Note that there is no halo surrounding the cocci-shaped cells.

RECIPES

Milk broth culture recipe
Modified from Handbook of Microbiological Media (2)

Composition per liter:
9.5 g of skim powder milk
Auto clave 15 minutes at 15 psi.

Litmus milk recipe
Handbook of Microbiological Media (2)

Composition per liter:
100.0 g of skim powder milk

0.5 g of Azolitmin
0.5 g of Na_2SO_3
pH 6.5

Autoclave for 20 minutes at 10 psi and 115 ^{o}C.

PROTOCOL FOR ANTHONY'S CAPSULE STAIN

A. General materials
- Staining tray
- Staining rack
- Slide holder
- Disposable gloves

B. Staining reagents

- Crystal violet 1% solution (primary stain)
- Copper sulfate 20% (decolorizer agent)

C. Procedure

1. Prepare a smear from a 12 - to 18 - hour culture grown in milk broth or litmus milk. (Serum protein may be used to prepare the smear if the organism was not grown in milk broth or litmus milk.) This is to provide a protei naceous background for contrast.

2. Allow the smear to air dry. DO NOT HEAT FIX (to avoid destroying or distorting the capsule or causing shrinkage).

3. Cover the slide with 1% crystal violet for 2 minutes.

4. Rinse gently with a 20% solution of copper sulfate.

5. Air dry the slide. DO NOT BLOT. (Blotting will remove the un - heat - fixed bacteria from the slide and/or cause disruption of the capsule.)

6. Examine the slide under an oil immersion lens. Bacterial cells and the proteinaceous backgrou nd will appear purplish while the capsules will appear transparent.

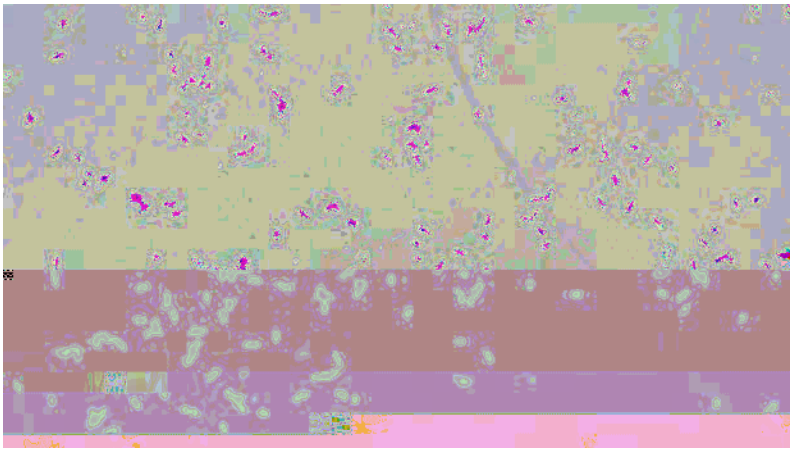

FIG. 6. Encapsulated Klebsiella pneumoniae grown in nutrient broth and stained using the Anthony's capsule stain. Note that no capsule is visible because no milk or other proteinace ous material was present or added to take up the crystal violet stain and provide a background.

FIG. 7. Encapsulated Serratia marcescens grown in skim milk broth and stained using the Anthony's capsule stain.

PROTOCOL FOR MANEVAL'S CAPSULE STAINING METHOD

A. General materials

- Staining tray
- Staining rack
- Slide holder
- Disposable gloves

B. Staining Reagents

· Congo red (1% aqueous solution)
· Maneval solution (see recipe or available from Carolina Biological Supply Company, NC)
· 0.05 g of fuchsin
· 3.0 g of ferric chloride
· 5 ml of acetic acid (glacial)
· 3.9 ml of phenol (liquified)
· 95 ml of distilled water

Prepare in a fumehood.

C. Procedure

1. Place a few drops of Congo red on a clean slide. Do NOT use a water drop in this sample preparation.

2. Mix in a small amount of culture.

3. AIR DRY. DO NOT HEAT FIX. (Heat fixing destroys protein caps ules; it coulTJ 0.o99 99hTJ.65 scn / off.16 4o6(O)-1m [(c)-2(ou)-1074.68s1( )1(a231m [(c)t1(o)9(n318(r)-12(e)

1. Be very careful when preparing the smear or capsule or slime will be lost. Do not shake the media; slime layers can be shaken off with vigorous shaking of the tube.

a “good bacterial sme ar” (hard to keep capsule intact).

3. One of the organisms used in the Capsule Stlll212.12 rhlle for

13. Roberts I. S. 1996. The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu. Rev. Microbiol. 50: 285 - 315
14.


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