Exercise 8.3 – Bile Esculin Test

LEARNING OUTCOMES

1. State the principle and procedure of the bile esculin test.
2. Explain how the bile esculin test is used to distinguish Group D enterococci from other streptococcal species.

The bile esculin test is used in the identification of Group D streptococci, including species of *Enterococcus.* Unlike many Gram-positive bacteria, enterococci grow in 4% bile, which serves as a selective agent in bile esculin medium. This medium also contains esculin, a carbohydrate derivative. While many organisms can hydrolyze or break down esculin, few other than the enterococci are able to do so in the presence of bile. When esculin is hydrolyzed, a molecule called esculetin forms and reacts with ferric (iron) ions added to the medium. This reaction causes a black precipitate and darkening of the agar (Figure 8.5).

Recall from earlier experiments that Group D enterococci are gamma hemolytic on blood agar, meaning that they do not act on red blood cells. Therefore, a positive bile esculin reaction is useful for distinguishing these bacteria from other catalase-negative, Gram-positive cocci.

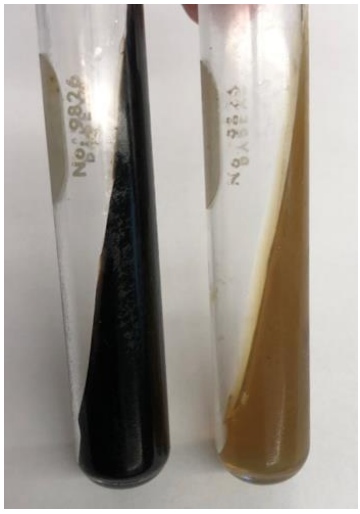

Figure 8.5: Enterococcus faecalis (left) hydrolyzes esculin in the presence of bile, forming a black complex of esculetin and ferric ions while growth of most other Gram-positive cocci is inhibited (right).

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OBJECTIVE

Distinguish Group D *Enterococcus* from other streptococci based on esculin hydrolysis in the presence of bile.

MATERIALS

- EQUIPMENT: Inoculating loop, incinerator
- MEDIA: Bile esculin agar slants
- CULTURES: *Enterococcus faecalis, Staphylococcus aureus*

PROCEDURE – STUDENTS WORK IN PAIRS

1. Use tape to label each tube with your initials, date, and organism number.
2. Aseptically inoculate bacteria on the slant surface in a single line from bottom to top.
3. Place tubes in a common rack for incubation at 37^{o}C for 18-24 hours.

FOLLOW UP

1. Evaluate tubes for esculin hydrolysis by observing blackening of the agar.
2. Record results in the lab report.
3. Remove tape from tubes and place them in a common rack for autoclaving.