DNase Test

Principle and Purpose

One virulence trait that most pathogenic strains of *Staphylococcus aureus* possess (and some other species, but not usually *S. epidermidis*) is the ability to produce the enzyme DNase. This enzyme, as its name suggests, degrades DNA. Other bacteria also produce this enzyme such as some streptococci species, most species of *Serratia* (Gram-negative bacillus) and *Moraxella* (Gram-negative coccus). DNase-negative bacteria include *Enterobacter* (Gram-positive coccus) and *Neiserria* (Gram-negative coccus), as well as the Gram-negative bacilli *Escherichia coli* and *Klebsiella.* Hence, this test is useful for distinguishing not only *S. aureus* from other staphylococcal species, but also between the Gram-negative cocci *Moraxella* and *Neiserria*, as well as *Serratia* from other species among the enteric bacteria.

The detection of DNase activity involves the use of media that incorporates DNA, but which may or may not contain a dye indicator (toluidine blue or methyl green; Fig. 1). Theses dyes are pH indicators. In this exercise, both media with and without dyes shall be used. The functional mechanisms of each is described below.

DNase test agar that contains a dye indicator will appear solid mint green (methyl green dye) or light blue (toluidine blue dye) in color. The color results from the combining of the dye with the DNA. When a microbe grown on this medium hydrolyzes the DNA, the complex is disrupted, and the medium changes color around the bacterial growth due to the change in acidity. If methyl green medium was used, a colorless (halo pattern) forms around the bacterial colonies as the medium becomes less acidic (Fig. 1). (DNA is an acid – deoxyribonucleic *acid*.) If toluidine blue-containing medium is used, the disruption of the dye-DNA complex results in a light pink

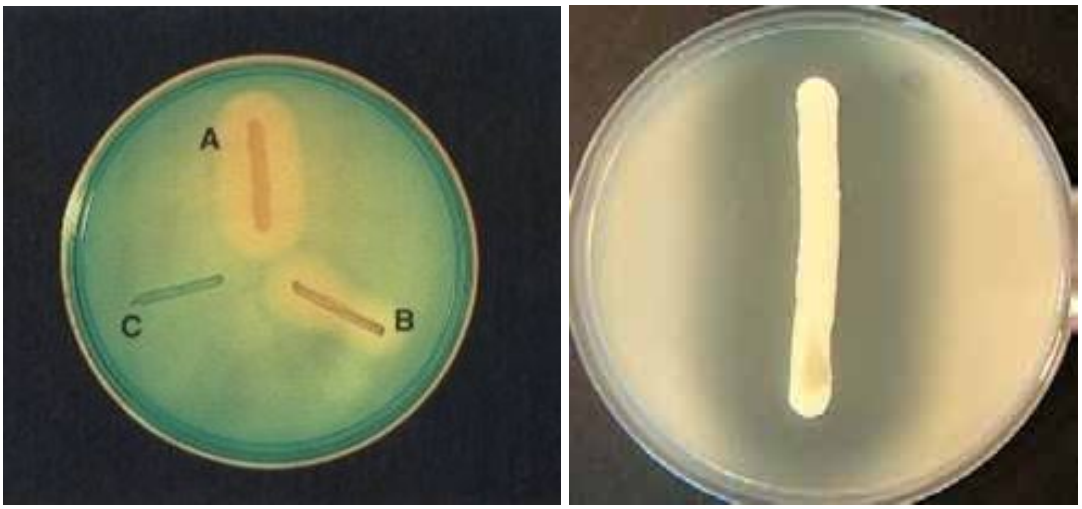


Figure 1. DNase Test Results Using Two Different Media. DNase agar containing methyl green (left image) inoculated with *Staphylococcus aureus* (A), *Serratia marcescens* (B), and *Staphylococcus epidermidis (C).* DNase agar without an indicator dye after HCl treatment (right image). The agar had been inoculated with *Staphylococcus aureus.* (https://microbeonline.com/deoxyribonuclease-dnase-test-principle-procedure-results/ and http://www.scharlabmagyarorszag.hu/katalogus/01-346_TDS_EN.pdf)

halo around the bacterial colonies (again, as the medium becomes less acidic). DNase-negative strains do not produce any type of halo in dye-containing media.

When DNase agar without indicator is employed, the hydrolysis of DNA can be observed after the addition of hydrochloric acid (HCl). HCl will dissolve any oligonucleotides remaining in the medium into DNA salts. These salts are insoluble. Hence, HCl causes unhydrolyzed DNA to precipitate, thereby making the medium opaque. Thus, DNase producing colonies hydrolyze DNA and produce a clear zone around the growth, whereas DNase-negative strains do not produce a clear zone (Fig. 1).

The following procedures should be performed using the same bacteria but with both dye-containing and non-dye-containing media. The dye-containing medium in this exercise employs toluidine blue as the indicator.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the underlying basis of the DNase test;
- Properly conduct the DNase test; and
- Accurately interpret the results of this test.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms - The following organisms should be provided as 24-48 hour-old TSB cultures:

- *Staphylococcus aureus* (ATCC 25923) [abbreviated as *S. aureus*]
- *Staphylococcus epidermidis* (ATCC 12228) [abbreviated as *S. epidermidis*]

Media

- DNase agar plate with toluidine blue
- DNase agar plate without dye

Reagents/Materials

- Sterile cottons swabs
- Sterile plastic bulb pipette
- 1N HCl
- Disposable gloves

Procedure

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain both a DNase agar plate containing toluidine blue and another that does not. Label them accordingly.
2) In addition, on the bottom of each plate, divide it in half labeling one side as *S. aureus* and the other as *S. epidermidis*.

3) Ensure that the bacterial cells in *S. aureus* broth culture are fully mixed by rolling the tube between both palms ten times or more to suspend any sediment of cells that may have formed. Roll the tube quickly, but not so harshly that the broth splashes onto the tube cap or such that the tube rolls out of the hands causing leakage or breakage.
4) Wet a sterile cotton swab by dipping it into the *S. aureus* culture, then gently pressing it against the side of the tube to remove excess liquid prior.
5) Using the swab wetted with *S. aureus*, aseptically make a broad, single streak on the appropriately labeled half of the toluidine blue plate.
6) Using the same swab, aseptically make a broad, single streak on the appropriately labeled half of the non-dye containing plate.
7) Repeat steps 4 through 6 to inoculate the other half of each plate with *S. epidermidis*.
8) Incubate both plates 18-48 hours at 37°C.
9) Remove both plates from the incubator.
10) For the toluidine blue plate, observe the area immediately surrounding each patch of bacterial growth.

Interpretation of Results: The presence of a clear, light pink zone around a patch of bacterial growth is indicative of DNase activity (see Fig. 1).

Record any observations on the data report sheet attached to this document.

11) For the non-dye containing plate, perform the following steps:

Note: The following steps use HCl, which is an acid that is best not to have on the skin. Wear gloves when performing these steps. If acid is splashed onto the skin, wash the area with soap and water.

a. Using a disposable plastic bulb pipette, gently flood the surface of the agar plate with a 1N HCl solution. Allow this to stand for 5 minutes
b. Drain off the excess HCl solution into the sink and follow this with flowing water for 1 minute.

Note: 1N HCl is bactericidal. Hence, after 5 minutes exposure, no viable bacteria are likely being placed down the drain of the sink.

c. Observe the plate for any clear zone around each patch of bacterial growth.

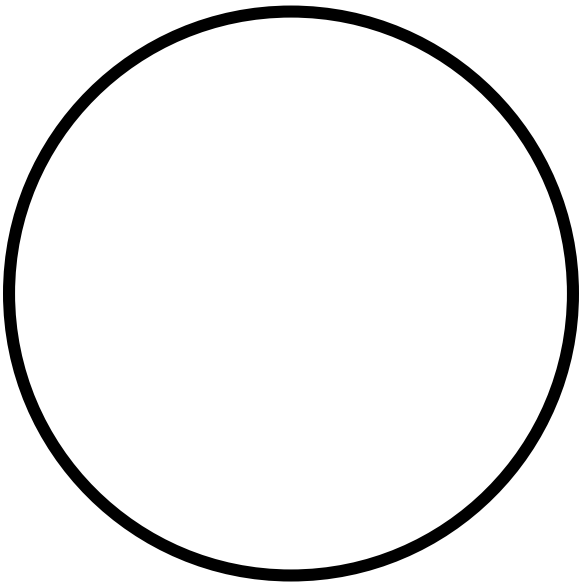
Interpretation of Results: The presence of a clear zone around a patch of bacterial growth is indicative of DNase activity (see Fig. 1).

Record any observations on the data report sheet attached to this document.

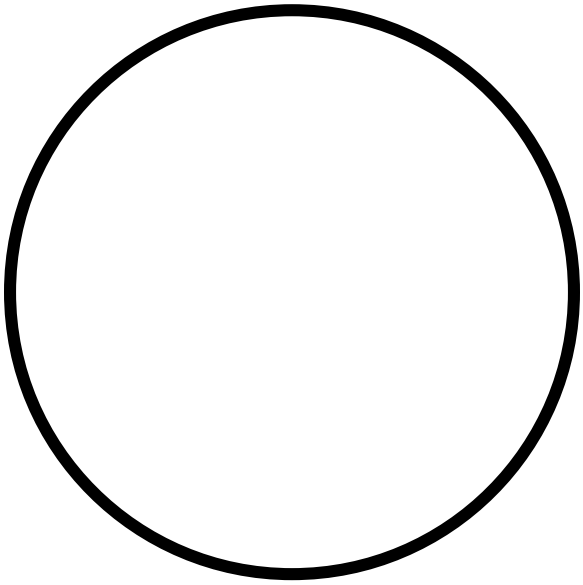
Student Name: _____

DNase Activity

Draw and label the observed results of DNase production by *Staphylococcus aureus* and *Staphylococcus epidermidis* on media with and without dye.



DNase Agar + Toluidine Blue



DNase Agar Without Dye

Literature Research

Using a published, *peer-reviewed* scientific literature source focused on the role of DNase by one or more microbes, summarize the findings of this article in 3-5 sentences to be written in the space below. Do not copy (plagiarize) the abstract or other content of this article! Use your own words/thoughts. In addition, attach a photocopy of the front page of this article to this report.