Lab 13: Bacterial toxins and enzymes

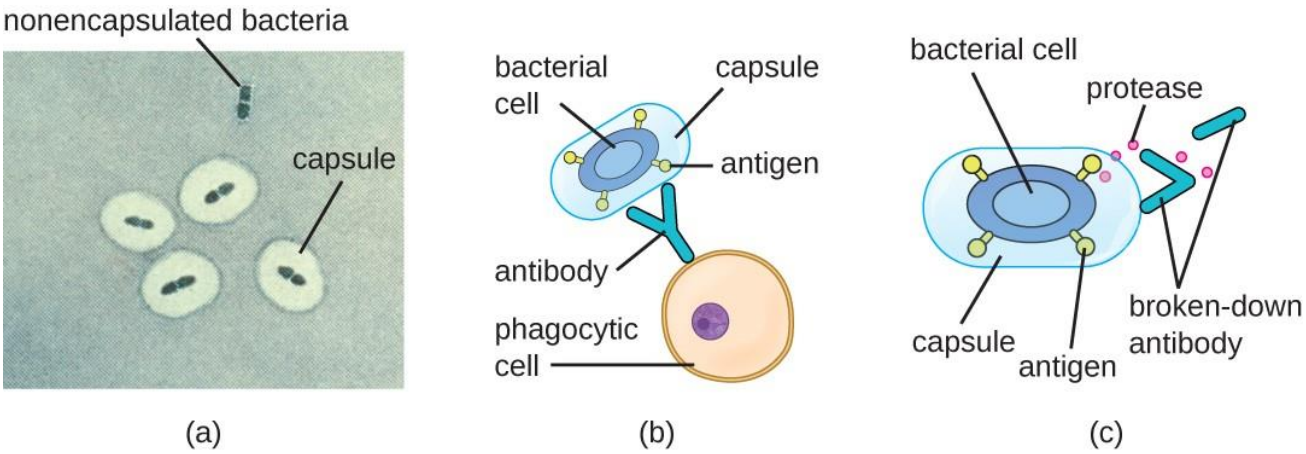


Figure 1: virulence factor for bacteria

Bacteria have many virulence factor that depend on it to attack the host cell that include capsule and many of virulence factor as in figure and secrete many of toxins and enzymes that damages the host cell , in this lab , we discuss some of toxins and enzymes produced by bacteria as follow :

1. Toxins

Many bacteria are able to produce poisonous substances called **toxins.** Toxins act on the body's cells, tissues, and organs and interfere with important body processes, thereby interrupting normal body functions. Those microorganisms that produce toxins are said to be **toxigenic.** The condition in which toxins are produced is called **toxaemia.**

Two important types of toxins are exotoxins and endotoxins. **Exotoxins** are proteins produced by bacteria during their growth and liberated into their surrounding environment. Exotoxins are produced chiefly by Gram-positive bacteria, and the genes for this production are carried primarily on the plasmids.

Various types of exotoxins exist. **Neurotoxins** interfere with the nervous system, while **enterotoxins** interfere with activities of the gastrointestinal tract. In response to

toxins, the body produces special antibodies called **antitoxins**, which unite with and neutralize the toxins, providing defense against disease.

It is possible to immunize against the effects of exotoxins by injecting **toxoids** into individuals. Toxoids are preparations of exotoxins chemically treated to destroy their toxigenicity but retain their ability to elicit antibody formation in the body. Toxoids are currently available to protect against diphtheria and tetanus (the DT injection).

Endotoxins are portions of the cell wall of Gram-negative bacteria. They consist primarily of lipopolysaccharides and are released when bacteria break apart during the process of lysis. Since lysis occurs during antibiotic therapy, the effects of endotoxins can bring about a worsening of symptoms during the recovery period. This condition is called **endotoxin shock.** It is accompanied by fever, chills, aches, and cardiovascular collapse.

1. Taxos A (bacitracin sensitivity testing)

This is a differential test used to distinguish between organisms sensitive to the antibiotic bacitracin and those not. Bacitracin is a peptide antibiotic produced by *Bacillus subtilis*. It inhibits cell wall synthesis and disrupts the cell membrane. This test is commonly used to distinguish between the haemolytic streptococci: *Streptococcus agalactiae* (bacitracin resistant) and *Streptococcus pyogenes* (bacitracin sensitive). The plate below was streaked with *Streptococcus pyogenes*; notice the large zone of inhibition surrounding the disk.

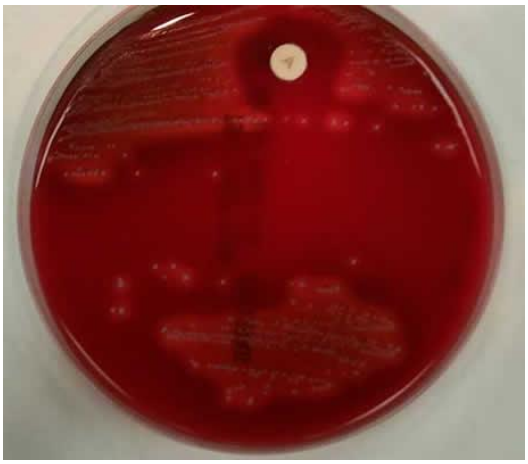


Figure 2: Bacitracin sensitivity testing for *Streptococcus pyogenes*

2. Taxos P (optochin sensitivity testing)

This is a differential test used to distinguish between organisms sensitive to the antibiotic optochin and those not. This test is used to distinguish *Streptococcus pneumoniae* (optochin sensitive (pictured on the right below)) from other haemolytic streptococci (optochin resistant (*Streptococcus mitis* is pictured on the left below)).

Figure 3: Optochin sensitivity testing for *Streptococcus mitis*

٢. Enzymes

Many pathogens produce a series of **enzymes** to help overcome body defenses and establish themselves in the host. One example is **leukocidins**, a group of enzymes that destroy white blood cells. This destruction lessens the body's ability to perform phagocytosis.

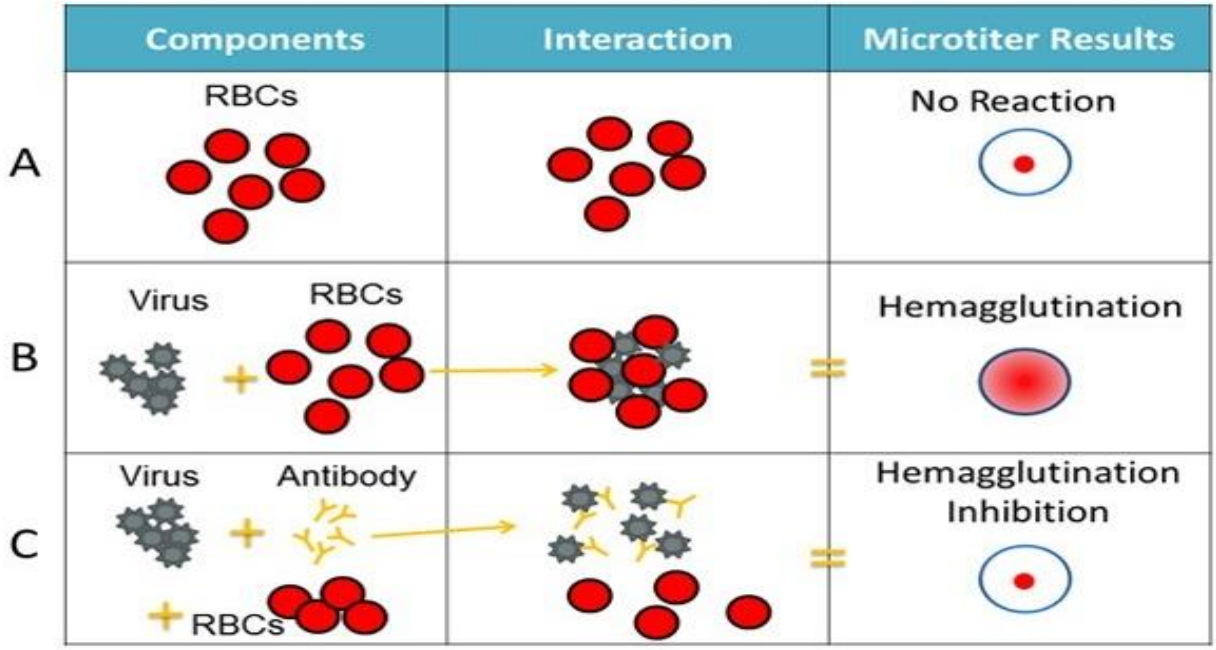
Other bacterial enzymes are **haemolysins.** These enzymes destroy red blood cells. Streptococci, staphylococci, and certain *Clostridium* species produce haemolysins.

Coagulases are bacterial enzymes that clot the blood. These enzymes convert fibrinogen into fibrin, which forms the threads of a blood clot. The clot helps staphylococci avoid the body's phagocytes and contributes to its pathogenicity.

Other important enzymes are streptokinase and Hyaluronidase. **Streptokinase** is a streptococcal enzyme that dissolves blood clots. This activity helps the organism escape the body's attempt to wall off an infection. **Hyaluronidase** destroys hyaluronic acid, a polysaccharide that "cements" cells together in a tissue. Hyaluronidase thus permits organisms to spread through tissues and establish themselves at sites distant from that of the initial infection. Another enzyme, called **collagenase**, breaks down collagen in the connective tissues of muscles. It thereby encourages the spread of infection.

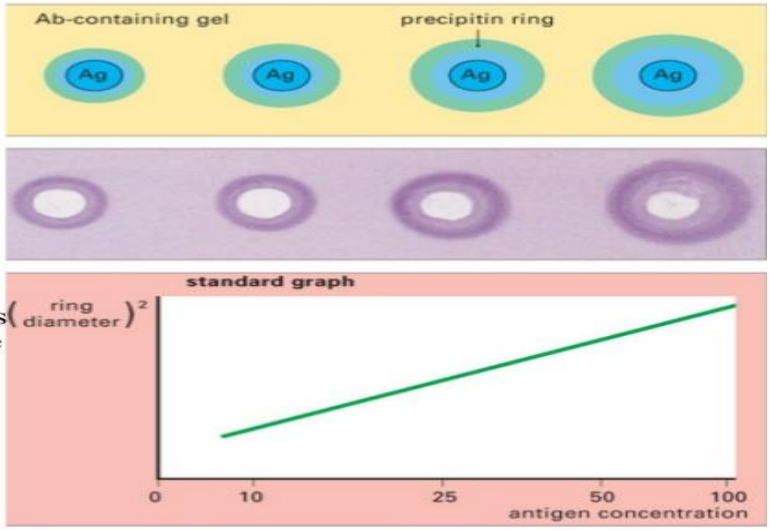
1. Haemagglutination test.

Two procedures based on haemagglutination have been described which are potentially more sensitive than immune-diffusion-based methods. Unlike the gel diffusion methods they do not require the antigen to be in a perceptible form. In the passive haemagglutination test, dilutions of sample are reacted with a constant amount of antibody. Toxin coated erythrocytes are then added and these agglutinate when there is free antibody present. In the reversed passive haemagglutination test, antibody is coupled to sheep erythrocytes and agglutination occurs when toxin is present.



Well	1	2	3	4	5	6	7	8	9	10	11	12
Dilution	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	Control
Pattern												

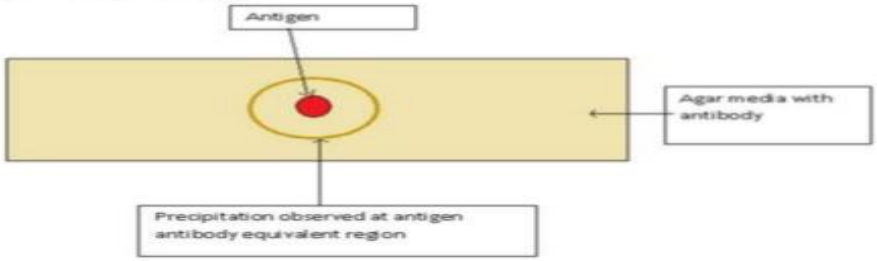
- *Method*
  - Ab in gel
  - Ag in a well
- *Interpretation*
  - Diameter of ring is proportional to the concentration
- Quantitative
  - Ig levels



Immune-diffusion-based methods

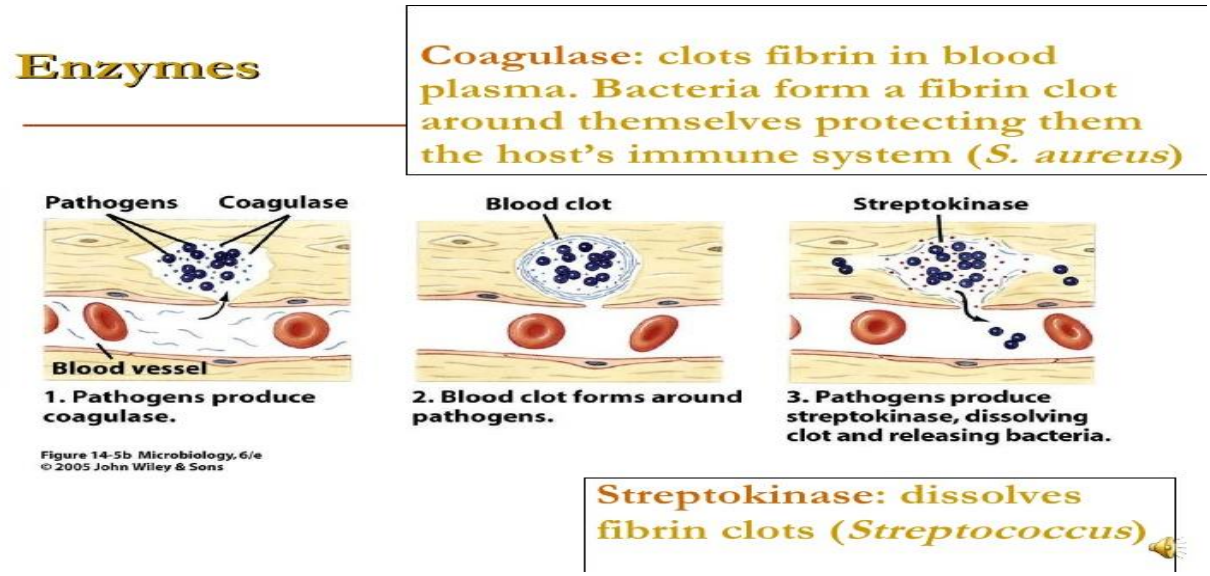
Gel - diffusion precipitation

The principle of the reaction: The antigen is placed in a well cut in an agar gel containing suitable diluted antibody. A ring of precipitate forms where the reactants meet in optimal proportions. The higher is the concentration of the examined antigen, the greater is the diameter of the ring. According to the diameter of the ring it is possible to count the concentration of the examined antigen.



2. **Conglutination test**

In this test, the antibody to the target toxin is coupled to the Protein A of non-viable *Staph. Aureus*. The Protein A binds to the Fc portion of the antibody, allowing the antigenic Fab sites to be freely exposed on the surface of the *Staph. Aureus* cells. The sensitized Staph. *Aureus* cells are mixed with the test sample on a slide and agglutinate within 10min if the target toxin is present. A commercial version of this test for *Salmonella*, *Shigella* and enter toxigenic *E. coli* is available .hylococcal infections."



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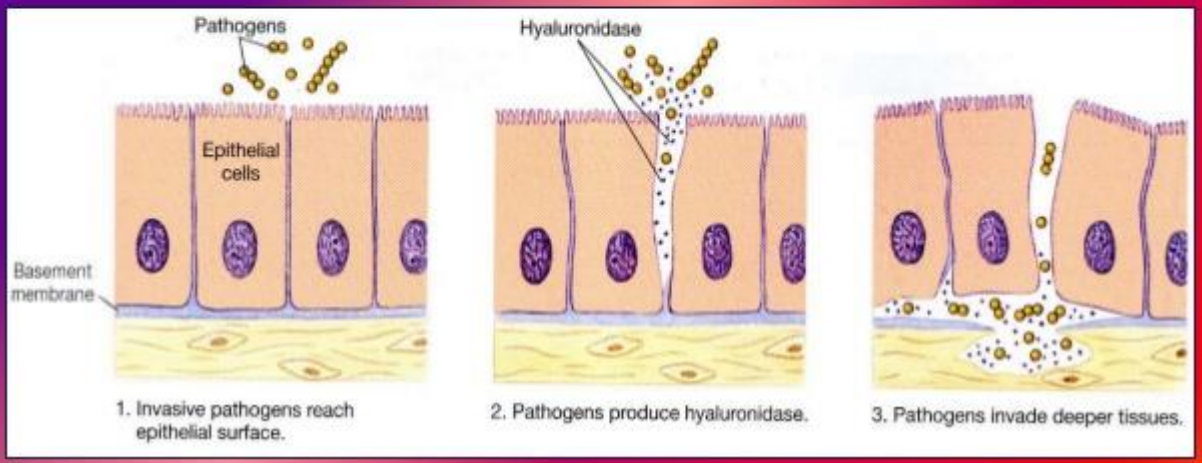
3. Hyaluronidase (Spreading factor)

Hyaluronidase digests hyaluronic acid, the "glue" that holds cells together



Collagenase

- Collagenase breaks down collagen, which is found in many connective tissues.
- Collagenase allows pathogens to spread through muscle tissue.
- Example:
- *Clostridium perfringens* - Gas Gangrene