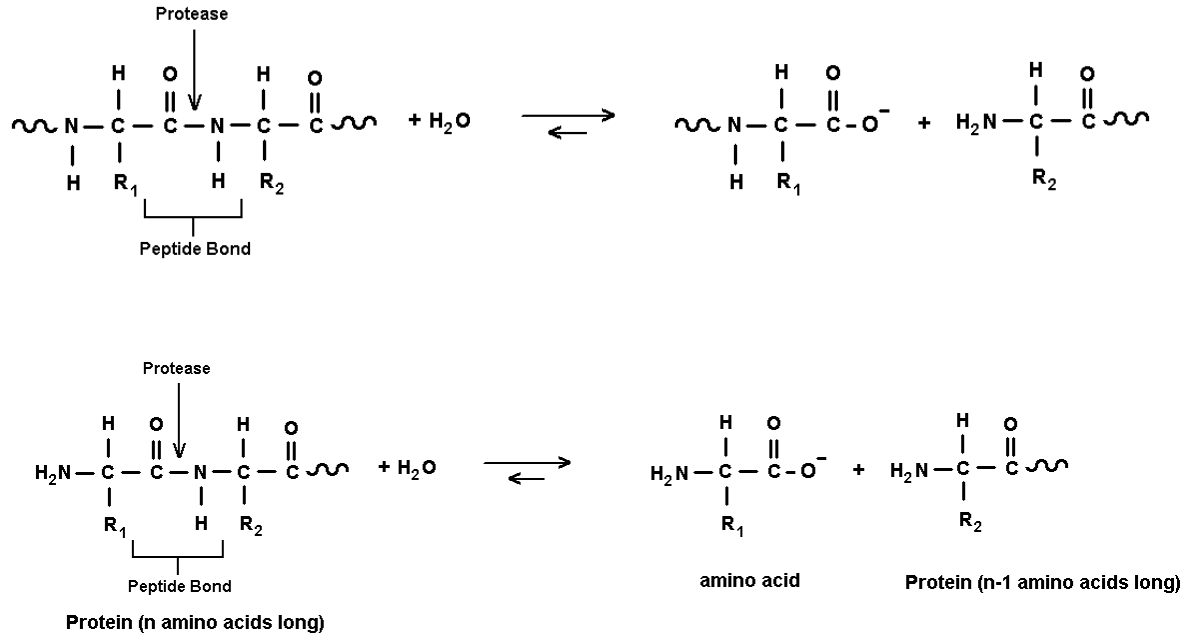


Gelatin Hydrolysis (Gelatinase Test)

When boiled in water, the connective tissue protein collagen (which is stringy, insoluble, and indigestible) changes into **gelatin**, a soluble mixture of polypeptides. Certain bacteria are able to hydrolyze gelatin by secreting a proteolytic enzyme called **gelatinase** (Figure 6-39 of the Atlas, p. 59).



The resulting amino acids can then be used as nutrients by the bacteria. Since hydrolyzed gelatin is no longer able to gel, it is a liquid. The ability of some bacteria to digest gelatin is an important characteristic in their differentiation (Figure 6-40 of the Atlas, p. 59). Since gelatin melts at 28 °C, it is sometimes difficult to distinguish a positive gelatinase test from simple melting. An alternative method to test for gelatinase will be used in this exercise.

Plate count agar (tryptic soy agar can also be used) is supplemented with 1.5% gelatin (1.5 g per 100 ml). Following inoculation of the agar plate and incubation, bacteria that secrete gelatinase will produce a zone of proteolysis which can be detected by adding a small amount of 15% trichloroacetic acid (TCA) to the plate. In the presence of trichloroacetic acid, proteins such as gelatin will become opaque, but hydrolyzed proteins will be clear (zone of clearing). A clear zone surrounding a colony (a positive reaction) is the result of a hydrolytic reaction that yields soluble amino acids. In a negative reaction, there is no gelatinase activity, and the medium surrounding the colony turns opaque upon addition of TCA.

Procedure:

To demonstrate the presence or absence of gelatinase activity we will use *Escherichia coli* and *Bacillus subtilis* as the test organisms.

First Period

1. With a marker, divide the bottom of a gelatin agar plate in half and label half the plate *Bacillus subtilis* and the other half *Escherichia coli*. Place your name and date on the

plate. Do not make a mark on the plate indicating where the bacteria will be inoculated because this may interfere with interpretation of the results.

2. Using aseptic technique, streak the respective bacteria onto the plate in a **straight line** within the section (see next page).
3. Incubate the plate in an inverted position for 24 to 48 hours at 35°C.

Second Period

1. Cover the plate with just enough 15% TCA to barely cover the plate. Use the TCA reagent sparingly. It is a strong acid so avoid contact with skin and clothing. Rinse exposed skin immediately with large volumes of water. Allow the plate to sit (right side up, lid up) on the bench for approximately 15 minutes. **Do not invert the plate.** Examine the plate for evidence of gelatin hydrolysis. Hydrolysis is evidenced by a non-opaque clear zone around the bacterial growth. If no gelatin hydrolysis has taken place, the zone around the bacterial growth will be opaque.
2. Record your results in the space below.

