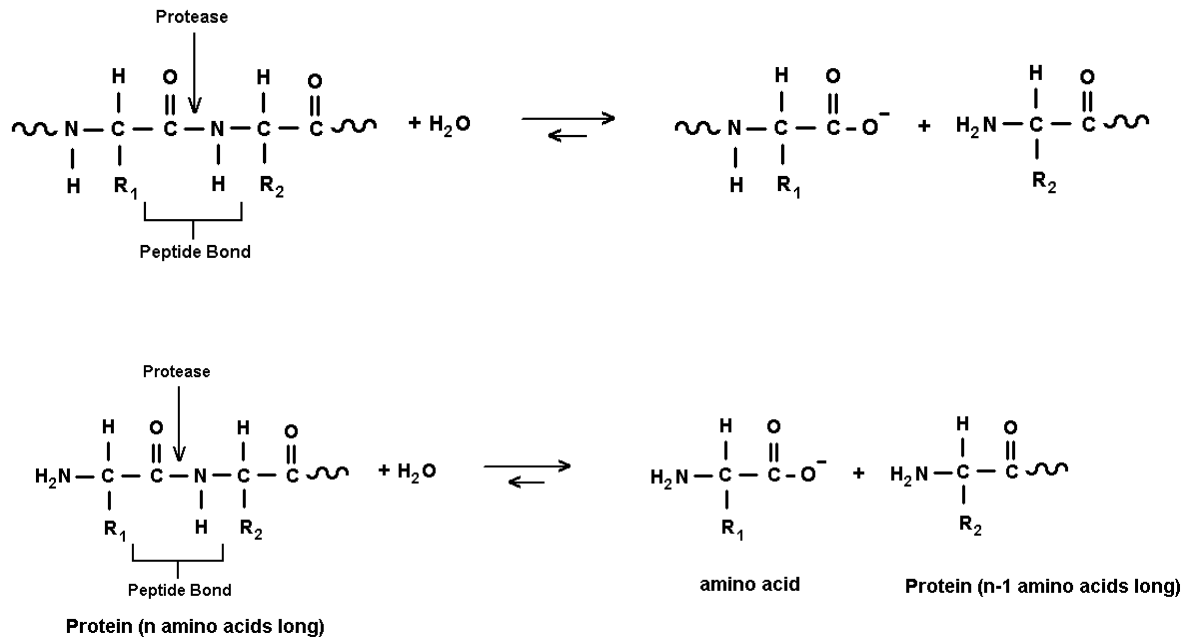


Casein Hydrolysis (Caseinase or Casease Test)

Casein is a large milk protein incapable of permeating the plasma membrane of bacteria. (Its presence is the reason milk is white). Therefore before casein can be used by some bacteria as their source of carbon and energy, it must be degraded into amino acids. Bacteria accomplish this by secreting proteolytic enzymes that catalyze the hydrolysis of casein to yield amino acids, which are then transported into the cell and catabolized.



When milk is mixed with plate count agar, the casein in the milk makes the agar cloudy. Following inoculation of the plate count agar, bacteria that liberated proteinases (e.g. caseinase, also known as casease) will produce a zone of proteolysis (a clear area surrounding the colony). Clearing of the cloudy agar (a positive reaction) is the result of a hydrolytic reaction that yields soluble amino acids (Figure 6-15 of the Atlas). In a negative reaction, there is no protease activity, and the medium surrounding the colony remains opaque (Figure 6-15 of the Atlas).

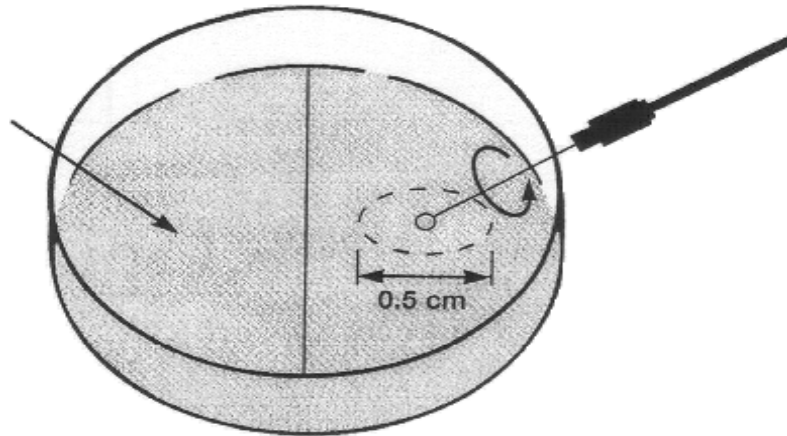
Procedure:

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

First Period

1. With a marker, divide the bottom of a skim milk agar plate (Plate Count Agar supplemented with 1% Skim milk; 1 % means 1 gram per 100 ml) in half and label one half of 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. Spot-inoculate the skim milk plate with the respective bacteria. Make a dime-sized circular spot of inoculum. (This circular pattern is preferred over the linear inoculation shown in Fig. 6-15)
3. Incubate the plate in an inverted position for 24 to 48 hours at 35°C.



Second Period

1. Examine the plate for evidence of casein hydrolysis (Fig. 6-15). Hydrolysis is evidenced by a zone of clearing around the bacterial growth. If no casein hydrolysis has taken place, the zone around the colony will remain opaque. [Depending on the source of skim milk powder, the opaque casein background of the plate may be hard to see. If the opaque casein background is hard to see, after incubation you can add 15% trichloroacetic acid to the plate. In the presence of trichloroacetic acid, proteins such as casein will become opaque, but hydrolyzed casein will be clear (zone of clearing)].
2. Record your results in the space below.