

NAME _____

DATE _____

Carolina LabSheets™

Bacterial Hydrolysis of Casein

Many bacteria secrete enzymes into their surroundings. These enzymes break down organic compounds, which are then absorbed by the bacteria and utilized as food. In this lab, you will streak two different bacteria onto skim milk agar, incubate the plates, and then observe the results. Casein is a protein that gives milk its white color. If the bacteria secretes protease, clear areas will develop around their growth, indicating the hydrolysis of casein.

Streaking Plates

Pick up a plate and mark the bottom to divide the plate into two equal sectors. Number the sectors 1 and 2. You will streak a different bacterium on the agar in each sector.

Mark the plate with your initials or group number and proceed to an inoculation workstation to streak your plate. You will streak the plate as follows:

Sector 1: *Bacillus subtilis*

Sector 2: *Escherichia coli*

Use the following procedure to streak the plate.

1. Pass the inoculating loop through the burner flame and allow it to cool. After flaming, do not put the loop down or allow it to touch any surface.
2. Raise the cover of the *Bacillus subtilis* plate culture enough to insert the loop.
3. Pick up a small amount of the bacterial culture on the loop, remove the loop, and close the lid of the plate.
4. Raise the cover of your marked petri dish just enough to insert the loop.
5. Streak the loop on the agar in Sector 1 of the dish. It is not necessary to dig into the agar. This will leave a streak of bacterial cells on the agar.
6. Remove the loop and close the dish.
7. Flame the loop again to kill the bacteria remaining on the loop.
8. Repeat steps 1 through 7, but this time streak Sector 2 with *Escherichia coli*. Remember to flame the loop when you finish.

Take your streaked plate to the location indicated by your teacher for incubation. Plates are incubated inverted, so that any condensation will fall onto the lid, not onto the agar surface, where it could cause contamination.

Observing and Recording Results

Record your observations of the growth and appearance of the two bacteria on skim milk agar.

Bacillus subtilis: _____

Escherichia coli: _____

Which, if either of the bacteria, secretes protease? How do you know?

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