Bacterial Transformation

Bacterial Transformation :is natural process by which the bacteria uptake foreign DNA from their surrounding environment.

Historical events

Transformation in bacteria was first demonstrated in 1928 by the British bacteriologist Frederick Griffith. Griffith was interested in determining whether injections of heat-killed bacteria could be used to vaccinate mice against pneumonia. However, he discovered that a non-virulent strain of *Streptococcus pneumoniae* could be made virulent after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent. In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They isolated DNA from a virulent strain of *S. pneumoniae* and using just this DNA were able to make a harmless strain virulent. They called this uptake and incorporation of DNA by bacteria "transformation". The results of Avery et al.'s experiments were at first skeptically received by the scientific community and it was not until the development of genetic markers and the discovery of other methods of genetic transfer (conjugation in 1947 and transduction in 1953) by Joshua Lederberg that Avery's experiments were accepted.

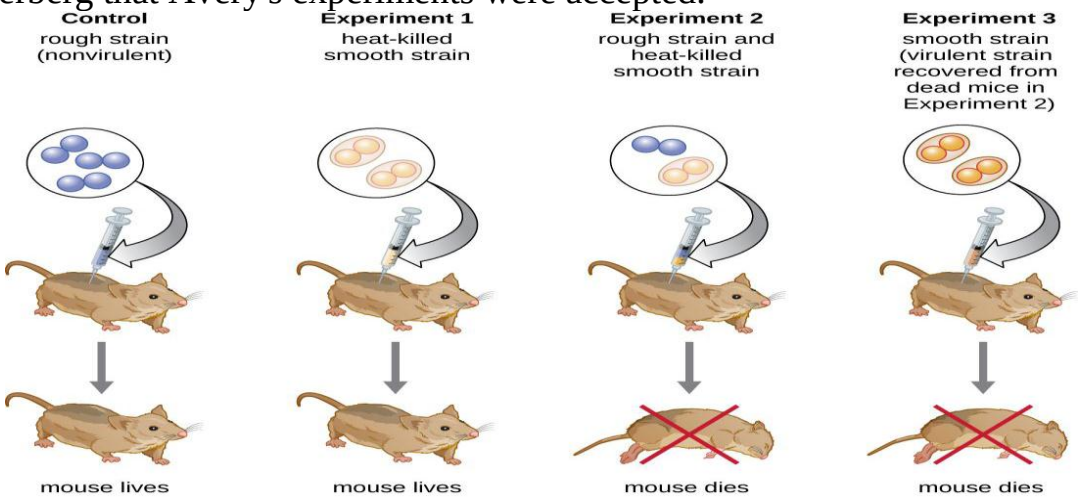


Figure 1: Griffith's experiment: Inject living encapsulated bacteria into mice.

Nowadays, many biomolecules can be produced by using transformed microorganisms. For example, patients suffering from diabetes mellitus require injections of insulin to control their blood glucose levels. As a large amount of insulin is needed by these patients all over the world, insulin is mass-produced from bacteria, such as *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (*S. cerevisiae*), that are transformed with the human insulin gene. Hence, bacterial transformation is an important and useful technique in the field of biotechnology.(Figure 2)

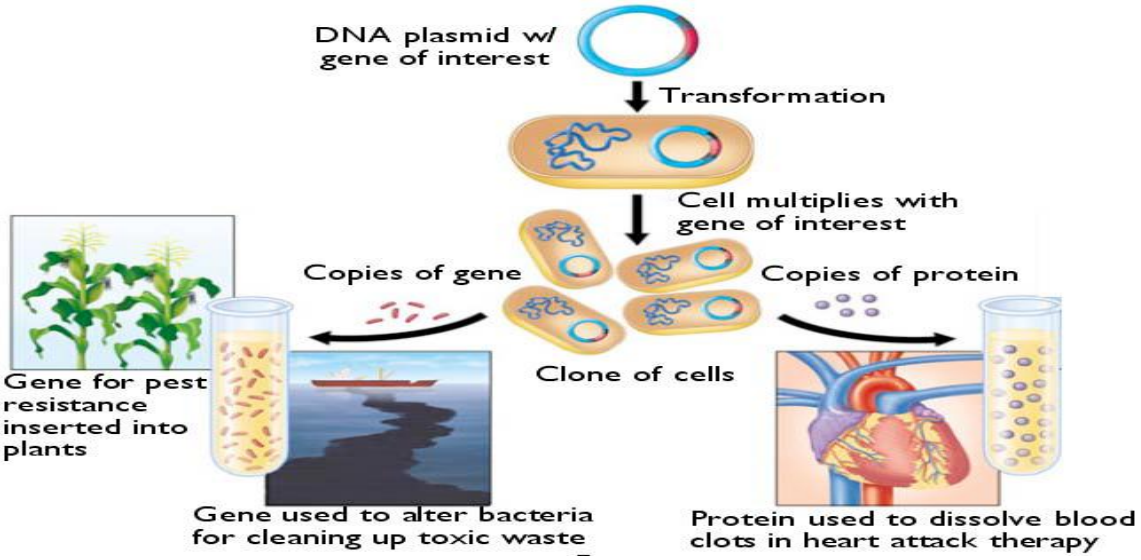


Figure2: Some benefit applications of bacterial transformation.

There are two types of bacterial transformation:

1-Natural transformation :

Transformation occurs naturally in some species of bacteria. For transformation to happen, bacteria must be in a state of competence, which might occur as a timelimited response to environmental conditions such as starvation and cell density. Some species, upon cell death, release their DNA to be taken up by other cells; however, transformation works best with DNA from closely-related species. These naturally-competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane.

2- Artificial transformation

Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA, by exposing it to conditions that do not normally occur in nature.

There are two methods of artificial transformation:

1-Electroporation: by using high voltage.

2-Chemical method: Typically, the cells are incubated in a solution containing divalent cations; most commonly, calcium chloride solution ($CaCl_2$) under cold condition, which is then exposed to a pulse of heat shock. The theory proposed for the creation of competency is rather recent and is based on the charges present on the phospholipids in the cell membrane as well as on the exogenous DNA molecule. Thus foreign DNA is normally repelled by the cell membrane, although there are pores (called adhesion zones) large enough in the cell membrane to allow the DNA to pass through. The addition of Ca^{+2} neutralizes the negative charges on both the phospholipid layer as well as the DNA and

cooling of the cells on ice congeals the lipid membrane. Sudden heat shock in the next step would increase the temperature outside the cell while it is still low on the inside. This temperature gradient would facilitate the movement of DNA into the cell.

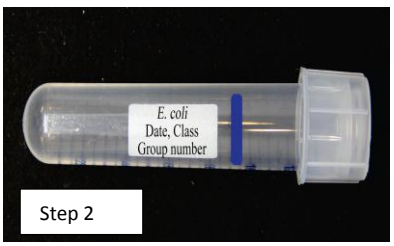
In this laboratory we will learn how to manufacture amylase using genetic transformation technique.

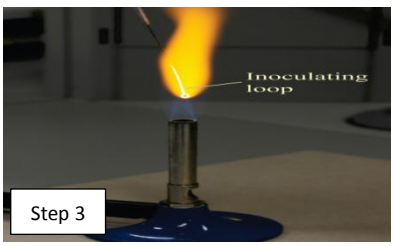
Procedure

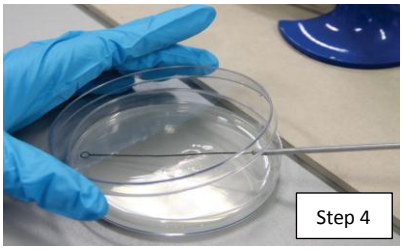
Part (1): Preparing *E. coli* cells for transformation.
Part (2):Transforming *E. coli* cells with a recombinant plasmid containing the amylase gene.
Part (3): Culturing *E. coli* cells on agar plates containing starch and ampicillin.
Part (4): Identifying transformed *E.coli* colonies.

Part (1): Preparing *E. coli* cells for transformation.

1.Disinfect the benchtop and gloved hands with 70% ethanol
2. Label the 5 ml LB broth culture tube "*E. coli*", with the date of the experiment, your class, and group number.
3.Flame the inoculating loop to red hot and allow it to cool down .
4.Raise the lid of the agar plate at about 45°. Put the inoculating loop onto the agar.
(Note: This is to minimize the exposure of agar to the atmosphere.)
5.Transfer the loopful of the *E. coli* colony directly into the 5 ml culture tube labelled "*E. coli*"
6.Cap the culture tube. Finger-flick the broth 2–3 times for a proper re-suspension.
7.Incubate the culture tube at room temperature or in an incubator set at 25−30°C for 48 hours.
8.After the suggested incubation time, store the tube at 4°C until the next lab .







Part (2): Transforming *E. coli* cells with a recombinant plasmid containing the amylase gene.

Equipment

- Micro-centrifuge for 2.0 ml micro-centrifuge tubes
- Water bath (42°C) - Ice bath
- Micropipettes and sterile tips

Materials

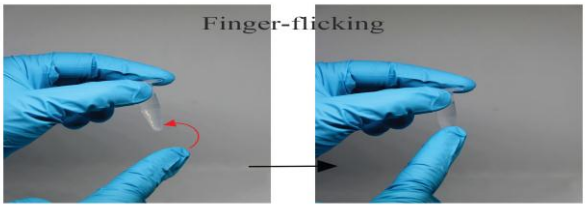
- *E. coli* broth culture from Part (1)
- Competent buffer (with CaCl2)
- pAmylase plasmid (0.08 µg/µl,)
- Sterile distilled water
- LB broth
- 1.75 ml micro-centrifuge tube
- Permanent marker
- 70% ethanol in spray bottle
- Paper towel
- Biohazard bag
- Disposal container with 10% chlorine bleach

Procedures

Replace LB broth with competent buffer

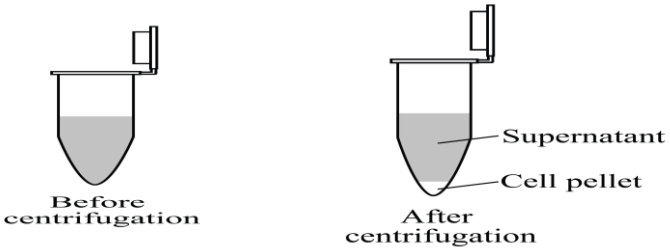
1. Disinfect the benchtop and gloved hands with 70% ethanol.
2. Label 2 micro-centrifuge tubes with your group number. Label one of them "pAmy" and the other one "C" (for the negative control).

3. Finger-flick the *E. coli* broth culture tube to re-suspend the cells. Use a P1000 micropipette and a new sterile tip to transfer 1.5 ml of this culture to each of the labelled micro-centrifuge tubes.

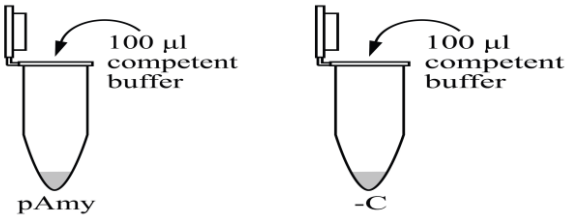


Note: The maximum volume of P1000 is 1000 µl or 1 ml. To transfer a volume of 1.5 ml, 2 draws of 750 µl are needed.

4. Centrifuge the 2 tubes at 5,000 rpm for 3 minutes to pellet the *E. coli* cells to the bottom . Carefully pour off and discard the supernatant in the designated disposal container with 10% chlorine bleach. **Save the cell pellet,** which is located at the bottom of the tube**.**



5. Using a micropipette with sterile tips, add 100 µl ice-cold competent buffer to each pellet. Gently pipette up and down a few times to suspend the pellet completely. Proceed to step 6 quickly



Note: Use a new pipette tip for each sample.
Note: Under normal conditions, DNA cannot pass through the cell membrane of bacteria. Calcium ions in the competent buffer help increase the permeability of the bacterial cell membrane, so that the plasmids can be incorporated.

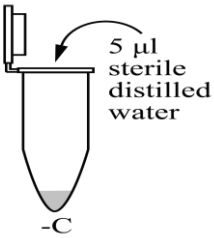
6. Centrifuge the 2 tubes at 5,000 rpm for 3 minutes to pellet the *E. coli* cells . Carefully pour off and discard the supernatant in the designated disposal container with 10% chlorine bleach. **Save the cell pellet.**

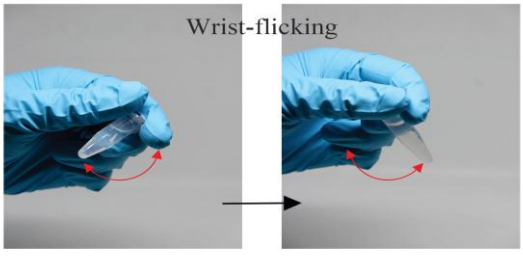
7. Using a micropipette with sterile tips, add 100 µl ice-cold competent buffer to each pellet. Gently pipette up and down a few times to suspend the pellet completely.

8. Gently finger-flick the tubes to avoid any cell sediment at the bottom of the tubes.
Note: Be gentle to these cells and do not vortex. They are fragile and can burst and die easily.

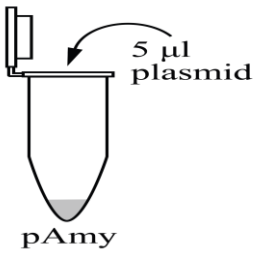
Transform plasmids into E. coli by cold shock and heat shock

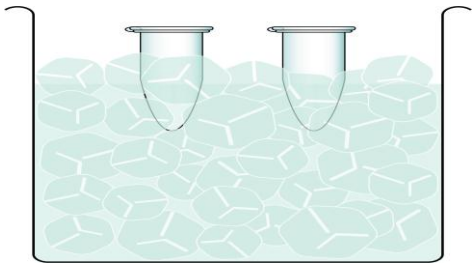
9. Keep all tubes on ice until Step 12.
10. Using a P20 micropipette and a new sterile tip, add 5 µl sterile distilled water to tube "C". Wrist-flick the sample to pool the reagents . **Return tube "C" to the ice bath.**



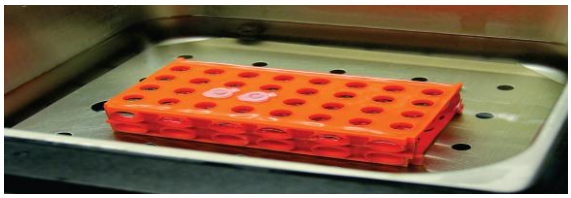


11. Using a micropipette and a new sterile tip, add 5 µl plasmid to tube "pAmy". Wrist-flick the sample to pool the reagents. **Return tube "pAmy" to the ice bath**.
12. Leave the tubes on ice for a minimum of 30 minutes .

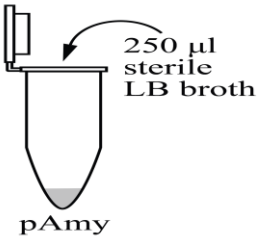


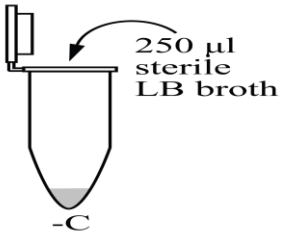


13. After 30 minutes, move the ice bath containing the tubes next to the hot water bath. Make sure the tubes are tightly closed. As quickly as possible, transfer both tubes from the ice to the 42°C water bath for a "heat shock" of exactly 90 seconds .



14. After 90 seconds, quickly return the tubes to the ice bath for 2 minutes.
Note: A sudden increase in temperature creates pores on the plasma membrane of *E. coli* and allows plasmid DNA to enter the bacterial cells. Transfer the tubes as quickly as possible, since the more distinct the temperature change in the heat shock, the greater the transformation efficiency.
15. Using a P1000 micropipette and new sterile tips, add 250 µl sterile LB broth to each tube . Mix the contents by finger flicking.
Note: Use a new pipette tip for each sample.



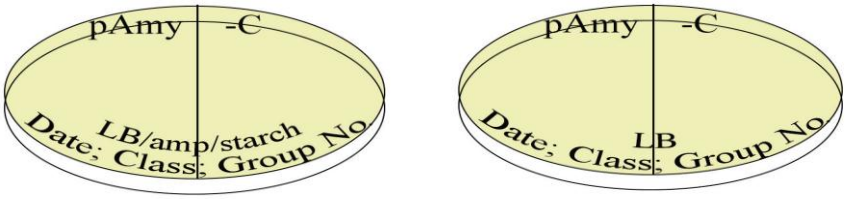


16. Incubate the tubes at room temperature or in an incubator set at 25–30°C for 45 minutes.
Note: Room temperature may vary, depending on the ambient conditions of the laboratory environment. Hence, the incubation time may also vary.
17. Discard the used tubes and tips in the designated disposal container with 10% chlorine bleach (no autoclave processing required) or in a biohazard bag for autoclave processing afterwards.

Part (3): Culturing *E. coli* cells on agar plates containing starch and ampicillin.

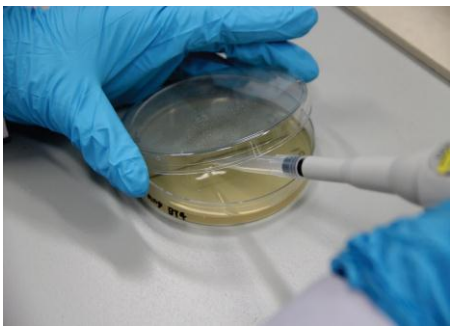
1.Disinfect the benchtop and gloved hands with 70% ethanol. Use a laminar flow hood, if available.

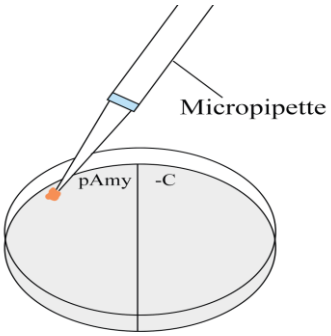
2.Obtain one LB/amp/starch agar plate (selective agar plate) and one LB agar plate (control agar plate). Label the bottom of the plates with the date of the experiment, your class, and group number. On the bottom of each plate, draw a line down the center. Label one side "pAmy" and the other side "C".



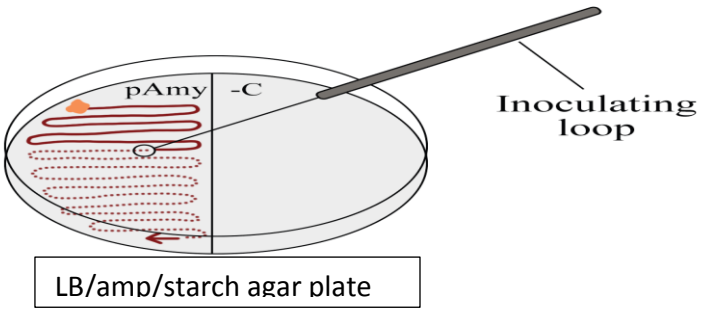
3.Collect the LB/amp/starch agar plate. Raise the lid of the agar plate at about 45˚.

4. Using a micropipette and a new sterile tip, transfer 30 µl *E. coli* culture from the tube "pAmy" onto the "pAmy" section of the LB/amp/starch agar plate. Discard the used tip in the designated disposal container with 10% chlorine bleach.



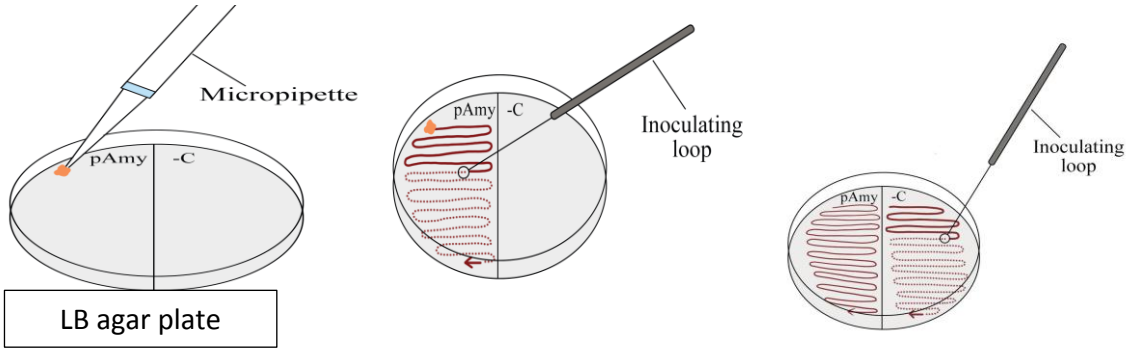


5. Streak the "pAmy" section with a sterile inoculating loop. Discard the disposable inoculating loop in the designated disposal container with 10% chlorine bleach.



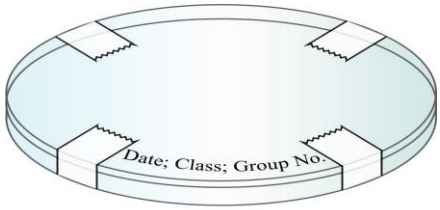
6. Using a micropipette and a new sterile tip, transfer 30 µl *E. coli* culture from the tube "pAmy" onto the "pAmy" section of the LB agar plate. Discard the used tip in the designated disposal container with 10% chlorine bleach.

7. Using a P200 micropipette and a new sterile tip, pipette 30 µl *E. coli* culture from tube "C" onto the "C" section of the LB/amp/starch agar plate. Discard the used tip in the designated disposal container with 10% chlorine bleach. Streak the "C" section with a sterile inoculating loop. Discard the disposable inoculating loop in the designated disposal container with 10% chlorine bleach.



8. Allow the culture suspension to be absorbed into the agar, by resting the plates on the bench undisturbed for 5 minutes, the lids facing up.

9. Stack the plates. Place short strips of adhesive tape at opposite edges of LB agar plates to prevent the plates from being accidentally opened.



10. Incubate the plates in an upside-down position at 4°C for 24 hours. Observe the results in Part (4) of the experiment within 3−4 days.

Part (4): Identifying transformed *E.coli* colonies.

Collect your group's plates. Observe the colonies on different agar plates. Take pictures of the plates, using a camera or mobile device.After observation and analysis, discard all unwanted culture plates in the designated disposal container with 10% chlorine bleach (no autoclave processing required) or in a biohazard bag for autoclave processing afterwards.

Results

1. Paste the photo or draw a picture of the LB agar plate (control agar plate)
2. Paste the photo or draw a picture of the LB/amp/starch agar plate (selective agar plate)
3. Describe and briefly explain what you have observed in the pAmy section of the LB/amp/starch agar plate.

Discussion

1-Why have we used an agar plate with starch in this experiment?

2. Suggest one method to show that the starch is digested by *E. coli*.

3. After the cold shock and heat shock, the tubes were mixed by "finger-flicking" and "wrist-flicking" instead of vortexing. Suggest the reason for this.

4. Suggest the function of adding ampicillin in the growth agar in this experiment.

Supported information for the experiment:

Amylase test (Starch hydrolysis)

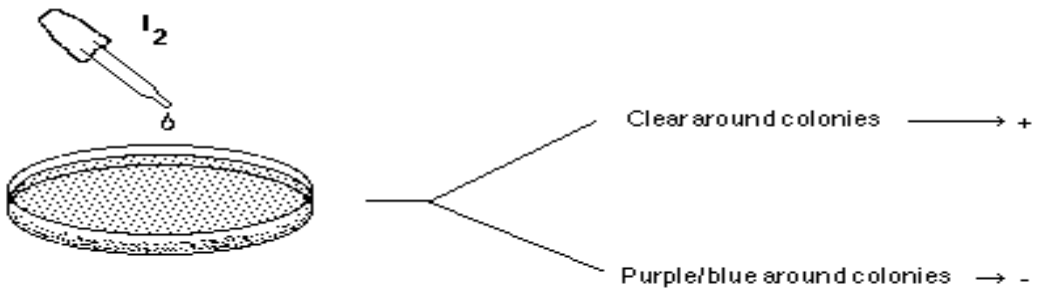
Objective: To determine if the organism is capable of breaking down starch into maltose through the activity of the extra-cellular α-amylase enzyme.

Test procedure

1.Use a sterile swab or a sterile loop to pick a few colonies from your pure culture plate. Streak a starch plate in the form of a line across the width of the plate. Several cultures can be tested on a single agar plate, each represented by a line or the plate may be divided into four quadrants (pie plate) for this purpose.

2. Incubate plate at 37 °C for 48 hours.

3. Add 2-3 drops of 10% iodine solution directly onto the edge of colonies. Wait 10-15 minutes and record the results.



Positive test (+): The medium will turn dark. However, areas surrounding isolated colonies where starch has been hydrolyzed by amylase will appear clear.

Negative test (-): The medium will be colored dark, right up to the edge of isolated colonies.