

Lab 6a: Bacterial Transformation

Introduction:

Bacterial transformation occurs when a bacterial cell takes up foreign DNA and incorporates it into its own DNA. This transformation usually occurs within plasmids, which are small circular DNA molecules separate from its chromosome. There can be 10 to 200 copies of the same plasmid within a cell. These plasmids may replicate when the chromosome does, or they may replicate independently. Each plasmid contains from 1,000 to 200,000 base pairs. Certain plasmids, called R plasmids, carry the gene for resistance to antibiotics such as ampicillin, which is used in this lab.

Plasmids function in transformation in two different ways. They can transfer genes that occur naturally within them, or they can act as vectors for introducing foreign DNA. Restriction enzymes can be used to cut foreign DNA and insert it into the plasmid vectors. The bacteria used in this lab were *Escherichia coli* (*E. coli*). It was ideal for this transformation study because it can be easily grown in Luria broth or on agar, and it has a relatively small genome of about five million base pairs.

Transformation is not the only method of DNA transfer within bacteria. Conjugation is a DNA transfer that occurs between two bacterial cells. A bridge is formed between the two cells and genetic information is traded. In transduction, a virus is used to transfer foreign DNA into a bacterial cell.

Hypothesis:

The transformed *E. coli* with the ampicillin resistance gene will be able to grow in the ampicillin plates, but the non-transformed *E. coli* will not.

Materials:

The materials needed for this lab were 2 sterile test tubes, 500 μL of ice cold 0.05M CaCl_2 , *E. coli* bacteria, a sterile inoculating loop, a sterile micropipette, 10 μL of pAMP solution, a timer, ice, a water bath, 500 μL of Luria broth, a spreading rod, 4 plates: 2 ampicillin+ and 2 ampicillin- , and an incubator.

Methods:

One sterile tube was labeled "+" and the other "-". A sterile micropipette was used to transfer 250 μL of ice cold 0.05M CaCl_2 to each tube. A large colony of *E. coli* was transferred with an inoculating loop to each tube. The suspension was then mixed by repeatedly drawing and emptying a sterile micropipette. 10 μL of pAMP solution was added to the cell suspension in the tube marked "+" and mixed by tapping the tube. Both tubes were immediately put on ice for 15 minutes and then soaked in a 42°C water bath for 90 seconds. The tubes were then returned to ice for another 2 minutes.

After the heat shock, 250 μL of Luria broth were added to each tube. The tubes were mixed by tapping. Two plates of ampicillin + agar were labeled LB/AMP+ and LB/AMP-. The two plates of ampicillin-agar were labeled LB+ and LB-. 100 μL of the cell suspension in the "+" tube were placed on the LB+ and the LB/AMP+ plates. 100 μL of the cell suspension in the "-" tube were added to the LB- and the LB/AMP- plates. These were spread with a spreading rod that was sterilized by passing it over a flame after each use. The plates were allowed to sit for several minutes and then incubated over night inverted at 37°C.

Results:

	LB+ (Positive Control)	LB- (Positive Control)	LB/AMP+ (Experimental)	LB/AMP- (Experimental)
Bacterial Growth	lawn	lawn	3 colonies	No growth

Questions:

1. Compare and contrast the number of colonies on each of the following pairs of plates. What does each pair of results tell you about the experiment?

LB+ and LB- Both of these plates had a lawn of bacteria. This proves that the bacteria are capable of growing on the agar and that there was nothing preventing growth beside the ampicillin.

LB/AMP- and LB/AMP+ The LB/AMP- had no growth, but the LB/AMP+ had small growth. This shows that the bacteria was transformed and developed a resistance to ampicillin.

LB/AMP+ and LB+ The LB/AMP+ had less growth than the LB+. This shows that the transformation was not completely effective and only transformed some of the most competent bacterial cells.

2. Total mass of pAMP used = 0.05 µg

Total volume of cell suspension = 510 µL

Fraction of cell suspension spread on the plates = 0.196

Mass of pAMP in cell suspension = 0.0098

Number of colonies per µg of plasmid = 0.0294

3. What factors might influence the transformation efficiency? Explain the effect of each you mention.

Transformation efficiency could be affected by the size of the colony added to the solution. In a larger colony, the efficiency would increase because more cells would be receptive. Another factor would be the amount of pAMP added. The more pAMP added, the higher the efficiency. The amount of Luria broth added could also affect efficiency. If the amount of Luria broth were increased, the efficiency would decrease.

Error Analysis:

This lab had several steps, each giving the potential for error. All of the measurements had to be precise and accurate, and the heat shock timing was a very complicated procedure. Error in this lab could have been caused by the concentration of the CaCl₂ due to the fact that most of it was frozen.

Discussion and Conclusion:

The bacteria treated with the pAMP solution developed a resistance to ampicillin and were able to grow on the ampicillin+ plate. Those that were not treated with the pAMP were not able to grow on this medium. The plates with no ampicillin served as a control to show how the bacteria would look in normal conditions. Transformation is never fully effective. Only cells that are competent enough are able to take up the foreign DNA. Therefore, the ampicillin + plates showed less growth than the control plate.