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Transformation of Bacterial Cells

We have all been told that DNA is the cellular blueprint and that it carries the instructions necessary to make each cell what it is. But how can we show that this is true? How can we convince ourselves that DNA is actually that important? In today's experiment, we will be artificially putting DNA into bacterial cells to see what effect, if any, this has on the growth or health of the cells.

How does this work? Normal, growing cells are not usually able to take up DNA (although this can occur under very specific conditions). We must chemically treat cells to create portals through which DNA can enter. After the DNA gets in, the cells are incubated under extremely favorable conditions to allow them to recover from this harsh treatment (imagine yourselves in Hawaii after finals and you'll be close).

What happens next? The bacterial cells will not distinguish this DNA as foreign and will use it as if it were their own. They will make copies of the DNA to pass onto future generations of bacterial cells and will also construct new proteins, as directed by the instructions on the foreign DNA.

How will we know this is actually happening? The cells that contain the new DNA (and, therefore, the new protein) will be able to do something they could not do before the DNA was added. A visible change will result that allows us to distinguish between treated and untreated cells.

The following section contains a more detailed, more technical explanation of what was briefly described above. It is meant to summarize what I will be discussing as we perform the experiment.

(Condensed from DNA Science; A First Course in Recombinant DNA Technology, D.A. Micklos and G.A. Freyer, editors, 1990.)

DNA, deoxyribonucleic acid, is the genetic material of the cell. Each gene carried on DNA performs a specific function in the cell. The DNA we will be using in today's experiment is called a plasmid. It is a small, circular DNA molecule that exists separate from the bacterial chromosome, but than can be copied many times by the bacterial cell and thereby passed along to daughter cells. In general, plasmids are not essential for survival of the host bacterium. Over the course of several generations, cells keep only those plasmids containing genes that improve their chances of survival under conditions that would normally kill the cells (those that provide a selective advantage).

Scientists can use the genes carried on plasmids as selectable markers – as tools to identify the cells that have taken up the plasmid DNA molecules. Characteristically, these marker genes are drug-resistance genes. Bacterial cells are normally sensitive to antibiotic drugs, such as penicillin and its derivative, ampicillin. All bacteria make cell walls to protect the cells from harmful substances in their environment. When bacteria divide, the cell walls break down and must be remade by each new daughter cell. The drug ampicillin blocks the synthesis of new bacterial cell walls. It does not affect cells with intact cell walls but kills dividing cells as they try to make new cell walls. The ampicillin

resistance (Amp^R) gene codes for an enzyme (called β -lactamase), which is able to destroy ampicillin. Cells that do not carry this gene are able to grow on normal nutrient media, but *not* on nutrient media containing ampicillin, whereas cells that *do* carry the Amp^R gene grow on both nutrient media and nutrient media plus ampicillin.

Transformation is the process by which the genetic content of a cell is changed. In the case of bacterial transformation, DNA is introduced into bacterial cells. Transformation occurs naturally among bacteria that are able to conjugate, a form of mating, but this is rare. Scientists are able to treat bacterial cells in such a way as to make them competent to be transformed in the laboratory. Treated cells that are able to take up foreign DNA are, therefore, said to be competent cells. Generally, cells can be made competent by suspending them in a solution of ice-cold divalent cations (typically Ca^{++} , although Mg^{++} and Mn^{++} have also been used), followed by a brief heat shock. Actively growing cells seem to give the highest percentage of transformants.

The mechanism of DNA uptake is not known, although there are plausible theories. One such theory suggests that the presence of pores in the cell wall allows the formation of channels through which DNA molecules can pass. These pores are present only in actively growing cells, which is consistent with the observation that they make the best competent cells. At $0^\circ C$, (ice-cold, the temperature at which DNA is introduced to the cells), the membrane is “frozen,” stabilizing the structure of the pores. DNA molecules and the phospholipids of cell membranes are both negatively charged. Electrostatic forces are therefore likely to prevent entry of the DNA into the cells. However, the divalent cations present in the cell suspension may interact with the negatively charged phospholipids, coating the pores. Channels into the cells are thereby created, lined with positive charges which attract the negatively-charged DNA molecules. The brief heat shock is believed to create a thermal imbalance between the inside of the cell and its external environment, which helps to “pump” the DNA into the cell. Cells are then grown at $37^\circ C$, which allows them to recover from the treatment, to make DNA, RNA and proteins, and to divide.

Today, we will be transforming 2 tubes of bacterial cells. To the cells in tube 1, we will add a buffer solution and to those in tube 2 we will add plasmid DNA. The cells in tube 2 will be transformed. The cells in tube 1 are mock-transformed and serve as a control – we will perform all the same manipulations on these cells as on those in tube 2, the only difference being that no DNA is added to tube 1. By doing this, we assure ourselves that any differences in the ability of the cells to grow do not arise from the differences in handling of the cells.

The plasmid we will be using contains the Amp^R gene, discussed above. Bacterial cells that are transformed by this plasmid will acquire the Amp^R gene and will make the enzyme that destroys ampicillin. So, they will be able to grow in the presence of ampicillin. Mock-transformed cells will not acquire or express the selectable gene. These cells will not be able to grow in the presence of ampicillin. However, both the transformed *and* the mock-transformed cells will grow quite happily on nutrient plates *without* ampicillin.

Experimental Protocol

1. You will be working with pure cultures of bacterial cells that you do not want contaminated with bacteria or fungi in the air. You will need to use sterile technique. Before starting the experiment, wash your hands and then wipe down your bench with 70% ethanol.
2. Each group will receive a pre-chilled, sterile tube (labeled either 1 or 2) that contains 50 μ l (0.05 ml) of competent cells. Keep these cells at 0°C (on ice).
3. If your group received a tube labeled Tube 1, you will perform the mock-transformation. Groups receiving tubes labeled Tube 2 will perform the actual transformation.
4. Using a pipettor with a sterile yellow tip attached, one student of each group will add 5 μ l (0.005 ml – a very tiny volume!) of sterile TE buffer to the cells in Tube 1. Move the pipet tip slowly through the cells while dispensing the liquid to gently mix it. To the cells in Tube 2, add 5 μ l of plasmid DNA. Replace the caps immediately after each addition.
5. Incubate your tube on ice for 20 minutes. Remember, this allows the negatively charged DNA to interact with pores in the cell wall that are lined with positively charged ions.
6. Heat shock the cells in your tube by placing them in a water bath at 42°C for 45 seconds. This helps the DNA to be drawn into the cells.
7. Place your tube on ice for 2 minutes.
8. Remove the cap from your tube and flame sterilize the mouth (pass through a flame briefly). Do the same to the small test tube labeled LB (Luria broth, a nutrient medium that allows the cells to grow). Pour the entire contents of the LB tube into the tube containing your cells.
9. Incubate the tubes at 37°C for 30-50 minutes. This allows the cells to recover from the cold and to begin to grow and make proteins before being exposed to ampicillin.
10. For all groups: One student will remove the cap from your tube and flame sterilize the mouth. Using a new sterile 1 ml pipet, the student will remove 0.1 ml of the cell suspension and transfer to an agar plate marked LB + Amp. A second student will sterilize the glass spreading rod by dipping it into 70% ethanol and holding it in a flame, allowing the ethanol to burn off. After briefly air-cooling the rod, the student will use the flat base to spread the 0.1 ml cell suspension uniformly over the surface of each plate.

Each group will repeat the procedure following the instructions above, except use a plate labeled LB (no ampicillin is present).

11. Allow the plates to dry at room temperature. After they have dried, invert the plates (so that if water condensation occurs, water will collect on the plastic lid, not on the agar) and place them in the 37°C incubator. They will grow there overnight.
12. The following morning, you or your teacher will remove the plates from the incubator. You should observe the plates sometime that day (or on another day, at your instructor's discretion). Count the number of white colonies that you observe on each plate.