Report on experiment Nr. 11

Bacterial Transformation

Group: D, Thursday

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Theory:

The Experiment was about the transformation of a pGlo plasmid containing *gfp* (*green fluorescent protein*) gene which was isolated from the luminescent jellyfish *Aequorea victoria* into an *E.coli* K-12:HB 101 strain.
A plasmid is a quite small circular DNA which carries e.g. resistance genes as well as operons.
In this case, the expression of the arabinose operon is relevant for the experiment. The DNA template for the relevant operon found in the HB 101 strains normally carries an arabinose operon with three functional genes (*araA, araB, and araD*) and one regulator gene (*araC*). For this experiment these three functional genes were cut out of the DNA template and are replaced by the luminescing GFP (Green-Flouroscent-Protein) gene from the jellyfish. The replacement in the HB 101 strain happens by taking inside the pGlo plasmid which has got the GFP gene on it and also encodes for an antibiotic resistance gene, that synthesises the enzyme beta lactamase which is resposible for the resistance against ampicilin.
Once the *E.coli* has taken up the pGlo plasmid, which is only possible when $CaCl_2$ is added to make the cells competent, i.e. permeable to large nucleic acid molecules. It is able to activate the luminescense only when the arabinose is present on the medium it is growing on. That is because the arabinose evokes a change of conformation in the araC protein which gives the bacteria (more specific: the template with the operon on it) the signal to start transcription of the GFP gene. Without arabinose the promoter gives the signal to not start transcription.
While incubating the cells for 24 hours, the resistance gene of the pGlo plasmid ensures, that no other colony but the recipient strain is growing on a medium which contains ampicillin.
As a result we expect the so produced colonies to have the ability to send out green light when excited by UV-A light. This is because of the three-dimensional structure of GFP, which is capabel to change from a higher level of energy to a lower one by emitting the loss of energy as green light.

Protocol:

While constantly working on ice, two Eppendorf tubes, labeled with (+)-DNA for the *E.coli* bacteria having the pGlo plasmid and (-)-DNA for those with no pGlo plasmid, were filled with the *E. coli* strain. They were centrifugated two times and each time the pelleted cells were resuspended with $CaCl_2$. The isolated plasmid was added to the (+)-DNA tube. Each

time the tubes were placed on ice for about 10 minutes. After putting the tubes in a bath of water (42°C) it was quickly changed back on ice. 250 µl of LB broth was added to each tube and afterwards 100 µl of each suspension was spead out on the appropriate plate. The plates contained as medium LB+Amp, LB+Amp+Ara for the (+)-DNA suspension (Amp stands for Ampicillin and Ara for Arabinose), as well as LB and LB+Amp for the (-)-DNA suspension.

Results and discussion:

The results were as follows:
The *E.coli* cells that had not come in contact with the pGlo plasmid were growing enormously on the LB-medium. That is because probably all of the cells that had been spead out on the LB plate just startet to grow as a normal bacterial culture. But there were none on the LB+Amp plate. That was because there was no resistance against ampicillin.
The bacteria on the other LB plate, that had taken up the plasmid were growing, but not as strongly as those on the LB plate, because not all of the bacteria brought on this plate had taken up the plasmid and with it the ampicillin resistance gene. Despite the growing, those bacteria did not light up, because arabinose was missing.
Finally those that were spead out on the LB+Amp+Ara plate also grew. However, they were the only bacteria which were shining up because of the presense of arabinose, as expected (see part „Theory“).