Report on Experiments 10 & 12

Group D, Thursday

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Introduction:

In this experiment, we worked with three different Bacteria strains:

- *E. coli* (pJL 1703): *tra* $^{-}$, *ina* $^{+}$, Kan ^{r}, Amp ^{s}, *lux* $^{-}$ (donor)
- *E. coli* (pRK 2013): *tra* $^{+}$, *ina* $^{-}$, Kan ^{r}, Amp ^{s}, *lux* $^{-}$ (helper)
- *V. harveyi*: *tra* $^{-}$, *ina* $^{-}$, Kan ^{s}, Amp ^{r}, *lux* $^{+}$ (recipient)

The donor provides the *ina* gene *(ice nucleation active*) in its plasmid, which makes the bacteria able to catalyse ice formation at temperatures of –2 to –12 °C. This gene makes an INA protein, which contains repeated amino acid domains of serin and threonin which are hydrophilic. This protein becomes the template for the water molecules, which will get oriented to form the ice.

But the donor cells do not have the *tra* genes (the plasmid is mobilizable but not *self-transferrable*), so they can't transfer the *ina* gene directly to the recipient. For this step, it needs the helper who's able to transfer the *tra* genes into the recipient. Therefore, the donor cells, first have to receive the *tra* gene from the helper, then they will be able to transfer the *ina* gene on a pJL1703 plasmid into the recipient. So the conjugation process needs all three bacteria strains in the same eppendorf tube to transfer the gene *ina*$^{+}$ into the *V. harveyi*. We tested this assumption in this experiment.

Methods:

We performed a *triparental mating* conjugation, where we prepared the different combinations of mixtures of recipient, donor and helper strains. After we had centrifuged the over night culture of the three bacteria strains (the Donor/D, the Helper/H and the Recipient/R cells) to collect a large enough amount of cells, we put them together in different mixtures: R alone, R + D, R+ H, D + H and also R + D + H.

Because the *cell to cell contact* is necessary for the conjugation process, the strains were then mixed together in a tube containing 1 ml LB, where the conjugation will take place. Now, our tubes with the mixtures and LB needed an incubation overnight by 30°C, so we carried on with plates from an other group. On each of the plates was one of the first four mixtures on a medium of LB, kanamycin and ampicillin. The mixture with all three bacteria strains was put on three plates in different concentrations.

On LB, all our bacteria strains we worked with are able to grow. But only *V.harveyi*, which got the transformation from the helper, has both antibiotic resistance genes. The *E. coli*, which have only the Kanamycin resistance gene (Kan^r), and the *V. harveyi*, which didn't get the plasmid from the helper, won't be able to grow on this medium. Now, our plates needed incubation for 48 hours by 30°C, so we could select where it will grow on plates from an other group. Only on the plates, where the mixture R + D + H was on it and where the conjugation processes have taken place, some transconjugant cells were able to grow. These bacteria were *V. harveyi*, which received the foreign genes from the donor with the *ina* genes and Kanamycin resistance gene. They also contain the *lux* gene, which we tested in the dark room. Each of us marked at least six colonies, which had biological luminescence, and put them on a plate with the medium LB, Kan and Amp. After a week, we took from each of this six sectors some cells and put them into six different glass tubes. We cooled them in a cooling bath and saw, that the water in our six tubes was frozen, but not the control glass tube, which contained only buffer solution without the bacterial cells. It was proven that these *V. harveyi* cells got the *ina* gene through a conjugation/genetic transfer, therefore the transconjugant cells were able to freeze water.

Luminescent bacteria:

These bacteria are wide spread in the marine environment, where they are present as planktonic forms and in symbioses. They live together with fish, squid, shrimp and other organisms, as parasites or as saprophytes.

Luminescence can have different functions like attraction of prey, intraspecies communication or escape from predators.

By luminescence, light production is catalysed by the enzyme luciferase. The figure 1 of experiment 12 shows, that it needs O_2 for the bioluminescence. In experiment 12, we tested the link between oxygen and luminescence. We filled a long glass tube

with a well grown culture of *V. harveyi*. On the top of the glass we left an air bubble. In the dark room, we turned the glass tube and saw, that the glowing started at the top of the glass tube where the oxygen was and got through it as fast as the oxygen could pass the solution with the bacteria. The same link was seen in a glass full of a suspension which contains *V. harveyi*. When we shoke the glass, the oxygen was mixed into the solution and the bacteria were able to make luminescence. But after a while, the light faded because the oxygen in the solution was used up.