Experiment 10

Bacterial genetic exchange

Conjugation of *ina (ice nucleation active)* gene from *E. coli* into luminous *V. harveyi*

Group D, Wednesday

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Introduction

Goal of the practicum is to understand a conjugation experiment (*triparental mating*) and to learn about *ina (ice nucleation active)* gene expression in recipient bacteria by performing the ice nucleation assay.

Practical Work

We got 3 tubes containing 2 strains of *E.coli* and 1 strain of *V.harveyi*.
Their genotypes were as follows:

1. *E. coli* (pJL 1703): *tra*$^-$, *ina*$^+$, Kan^r, Amp^s, *lux*$^-$ (donor/D)
2. *E. coli* (pRK 2013): *tra*$^+$, *ina*$^-$, Kan^r, Amp^s, *lux*$^-$ (helper/H)
3. *V. harveyi*: *tra*$^-$, *ina*$^-$, Kan^s, Amp^r, *lux*$^+$ (recipient/R)

To get a pellet of our bacteria we centrifuge them. Then we mixed the bacteria with 100 µl LB medium with the following treatments: R, R+D, R+H, D+H, R+D+H
We transferred our mixtures in 5 Eppendorf tubes with 1ml LB Agar and incubate over night at 30°C.
We took the tubes (except R+D+H) from the incubator and transferred the cells on Agar-plates containing LB, Kan and Amp. In the R+D+H-tube we added 0.9 ml LB containing Kan and Amp. We then transferred 25 µl, 50 µl and 75 µl on 3 different Agar-plates also containing LB, Kan and Amp. We incubated all the plates for 48 h at 30°C.
After 48 h we took the R+D+H-plate and detected the luminescence colonies in the darkroom. We randomly selected 12 of those colonies and put them on 3 new Agar-plates which are divided in 6 sectors. We incubated over night at 30°C.
We transferred 2 loops of each grown colony into 12 test tubes containing 10 ml of sterile PBS. Then we put them into a circulating cooling bath at -5°C for 10 minutes. As controls, we also tested the solution without cells (as negative control), the solution containing donor cells (positive control) and recipient cells (negative control).

Results

- On the plates with R, R+D, R+H, D+H nothing had grown.
- The R+D+H plate had many colonies which were luminescence in the darkroom.
- The tested tubes containing transconjugant cells were all frozen.

Discussion and Background

- R didn't grow because it is sensitive to Kan.
- R+D didn't grow because without helper the conjugation didn't take place (because the donor has no *tra+* gene which is responsible for making the sex-pili)
- R+H didn't grow because only the donor can conjugate with the *V.harveyi*.

- D+H didn’t grow because they’re both sensitive to Amp.
- R+D+H is glowing and making ice because the helper gave his *tra*+ gene to the donor so it could make a sex-pilus and transfer its *ina*+ gene to *V. harveyi*. So the transgenic bacteria can glow and make ice because they have all the genes for it. Unfortunately the ice itself cannot glow because it needs oxygen, substrates and enough active *V. harveyi* cells which express luciferase genes. If this would have occured, it would have been very nice.