Experiment 10

Bacterial genetic exchange: Conjugation of *ina (ice nucleation active* gene from *E. coli* into luminous *V. harveyi*

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Introduction: For goals and experiences gained look at the instructions in the course handout.

Practical Work: We get 2 tubes with *E. coli* and 1 tube of *V. harveyi* with the genotypes:
E. coli (pJL 1703): tra$^-$, ina$^+$, Kan^r, Amp^s, lux$^-$ (**donor/D**)
E. coli (pRK 2013): tra$^+$, ina$^-$, Kan^r, Amp^s, lux$^-$ (**helper/H**)
V. harveyi: tra$^-$, ina$^-$, Kan^s, Amp^r, lux$^+$ (**recipient/R**)
We fill 3 Eppendorf tubes with 1ml D, 3 with 1ml H and 4 with 1ml R each. Then we put them 2 min. in the centrifuge at 7000 rpm and pour off the supernatant.
4 treatments are controls; R, R+D, R+H and D+H each mixed with 100µl *LB*, transfered into 1ml *LB*-agar, incubated overnight at 30ºC and then spread on agar plates (*LB, Kan, Amp*), next incubation for 48 hours at 30ºC.
We give 100 µl *LB* into the tube with R mix it and put the liquid into the tube with D and then into the tube with H. At the end we transfer it into a tube with 1 ml *LB*-medium and incubate it overnight at 30ºC. After this we dilute with 0,9 ml *LB* medium containing Kan and Amp and take 25µl, 50µl and 75µl to spread out on agar plates (*LB, Kan, Amp*), next incubation for 48 hours at 30ºC. In the dark we mark some luminescent colonies and put them on new agar plates (*LB, Kan, Amp*) that we get enough cells for the ice nucleation assay, for which we take cells and transfer them into PBS. We put the test-tubes in a cooling bath for 5 min. And look if it freezes.

Results/Discussion: Nothing grew on the control agar plates: R is sensitive to *Kan*, R+D make no conjugation because D has *tra-* and D is sensitive to *Amp*, R+H make conjugation but the plasmid isn't working in *V. harveyi*, D+H make conjugation but they are both sensitive to *Amp*.

We see luminescence on the agar plates and also the freeze test is positive. So we can say that there is conjugation in some cells in the test-mixture (R+D+H), where the plasmid of H goes into D and helps that both plasmids can get into R. These new cells are resistant to *Kan* and *Amp* and have *ina+*.

Appendix: http://www.microeco.unizh.ch/uni/kurs/bio3_04/start/framepg1-css-b.html
and
http://www.microeco.unizh.ch/uni/kurs/bio3_04/docs/ex-10.html
for the experimental protocols
Reading Chapters 9.1, 9.5, 9.9 in BBOM 9th