Experiment 10

Bacterial genetic transfer: Conjugation of *ina* (*ice nucleation active*) gene from *Escherichia coli* into luminous *Vibrio harveyi*

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Introduction:

The main goal of this experiment is to demonstrate gene transfer by bacterial conjugation, mediated by plasmids.The experiment was done by performing a triparental mating.
It was our goal to transfer the *ina* gene from *Escherichia coli* into a wild type, luminous *Vibrio harveyi*.
The *ina* gene catalizes ice nucleation formation at temperatures between –2 to –12°C, much higher than organic substances freez under normal conditions. In plants this ice formation causes frost injury (which makes organic substances available for the bacterial cells and thus enhances infections).
V. harveyi is a bacterium occuring in shrimp and in or on many marine organisms, it is luminescent which makes it easy to detect in the dark.

Practical work and Results:

As a donor (D) we used *E. coli* carrying a plasmid (pJL 1703) encoding the *ina* gene. This plasmid carries a kanamicin resistance, as an antibiotic marker. The plasmid is mobilizable but it isn't self-transferable because it lacks a *tra* (transfer) operon.
Therefore, we needed a helper (H) strain *E. coli* (pRK 2013) which provides the *tra* gene and which is also kanamycin resistant. This helper has a highly specific suicide plasmid, which inhibits it from being expressed in other bacteria, which do not belong to the same genus. As the recipient (R) we used a wild type, luminous, *ina* deficient *V. harveyi,* which is resistant to Ampicillin, but sensitive to kanamycin.
In the experiment we prepared a mixture of donor, helper and recipient in an Eppendorf tube with sterile LB, in which cell-to-cell contact of the conjugation processes were supposed to take place. As controls we prepared also the following treatments R, R+D, R+H, D+H.
Afterwards, we incubated the tubes overnight at 30°C. The next day, we diluted the RDH mixture with LB containing Ampicillin and Kanamcin. We took different dilutions of the RDH mixture, and spread the cell suspension on petri dishes on LB agar containing Amp and Kan. Controls were plated undiluted on LB agar with Kan and Amp.
Then we incubated again for 48 hours at 30°C. After this incubation 18 colonies of luminescent transconjugant bacteria (tra^-, ina^+, Kan^r, Amp^r, lux^+) were choosen (in the dark room) and then spread again on LB agar plates with Kanamicin and Ampicillin. After overnight incubation the bacteria were diluted in sterile PBS, then we put the test tubes in the circulating cooling bath (at –5°C) for 10 minutes. As a control, we used sterile PBS without bacteria. After 10 minutes the PBS containing the bacteria was frozen, while the PBS without the bacteria remained fluid.

Further readings:

Madigan, M.T. et al. (2003): Brock - Biology of the Microorganisms, 10th ed. Chapters: 9.1, 9.5, 9.9, 10.1, 10.5, 10.9
Gurian-Sherman, D. et al. (1993): Bacterial Ice Nucleation: Significance and Molecular Basics, FASEB

Experiment 11

Bacterial genetic exchange: Transformation

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Introduction

The main goal of this experiment is to demonstrate gene transfer by bacterial transformation, which was first discovered by Griffith (1928).
The Green Fluorescent Protein (GFP) gene was modified by mutagenesis and inserted into the pGlo plasmid that encodes the gene for a beta lactamase (*bla*), which provides resistance to the antibiotic Ampicillin. The GFP gene can be switched on in transformed cells simply by adding the sugar arabinose (Ara) to the nutrient medium. *Escherichia coli* K-12:HB101 was used as the recipient strain and plasmid DNA (pGlo) was transformed into these cells.

Procedure

For the transformation of pGlo into *E. coli* we took an overnight culture of *E.coli* HB101 and incubated 1ml in 10ml LB (Luria Bertani medium) for three hours at 37°C. At this point we kept all samples on ice. By centrifugation we collected *E.coli* cells from the suspension and mixed them with $CaCl_2$ to make the host cell competent. After 15 minutes we collected the cells again and divided them into two Eppendorf tubes. In one we put pGlo plasmid (+pGlo) solution and incubated both (+pGlo, -pGlo) for 10 min on ice. The Ca^{2+} ions neutralize the negative charged phosphate groups of the plasmid DNA and facilitate the entry of the DNA into the *E.coli* cell (uncharged particles diffuse better through the membrane).
After incubation on ice we put the tubes in a floating rack quickly into a 42°C waterbath incubator for a heat-shock during 50 seconds, which is important for a successful transformation. After this step we put them back into the ice.
We incubated the tubes again by room temperature with LB medium for 10 min. Four prepared agar plates were labeld on the bottom as follows:

- LB+Amp (+pGlo)
- LB+Amp+Ara (+pGlo)
- LB+Amp (-pGlo)
- LB (-pGlo)

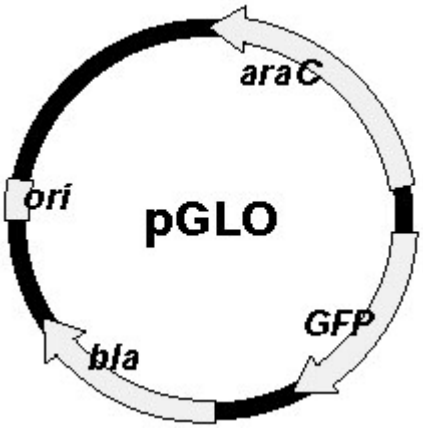
After spreading the suspensions on the agar plates we incubated them at 37°C during night.
(For more precise instructions: http://www.microeco.unizh.ch/uni/kurs/bio3_03/pdf/11transfo02.pdf)

Results

	- pGlo		+ pGlo	
	Growth	Luminescence under UV light	Growth	Luminescence under UV light
LB	+++	-		
LB Amp	-	-	+	-
LB Amp Ara			+	+

+++ Good growth (cell lawn) because of optimal conditions (no antibiotics)
+ Not every cell has taken up a pGlo plasmid and so only single cells were Ampicillin resistant and could grow.

Discussion



The arabinose operon consists of three genes.
Transcription of these three genes requires the presence of the DNA template (promoter and operon), RNA Polymerase, an AraC (DNA binding protein). When arabinose is absent the RNA Polymerase cannot bind to the DNA binding site and no protein is transcribed.
In the pGlo plasmid the three genes have been replaced by a single gene, which codes for the GFP. In the absence of arabinose, the GFP gene is not transcribed and the colonies are not fluorescent.

Appendix

BBOM 9th: 9.6, 10.16, 7.2
BBOM 10th: 10.6, 31.1, 8.5, 20.8
Illustration: http://www.gunn.palo-alto.ca.us/~ghorsma/rainforest/pGLO.JPG

Experiment 12

Bioluminescence of *Vibrio harveyi* (demonstration)

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Introduction

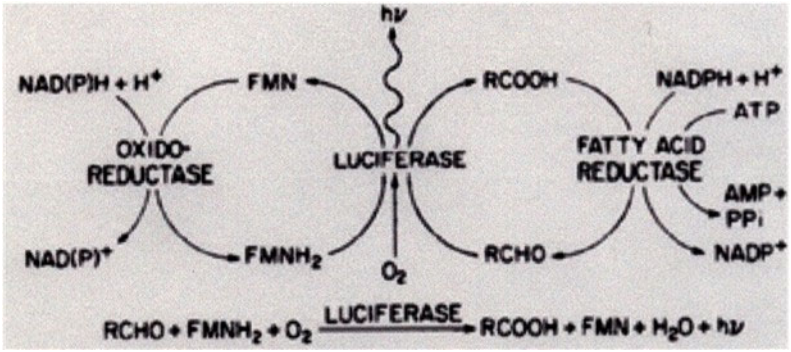
Vibrio harveyi is a luminescent bacterium. Light production is catalyzed by the enzyme luciferase. Luciferase catalyzes the oxidation of a reduced flavin and a long-chain aldehyde, producing oxidized flavin and the corresponding long chain fatty acid. Expression of the genes for luciferase (*lux*) occurs in late log phase and was shown to be induced by a small sensory molecule called autoinducer. Because of autoinduction free-living luminescent bacteria in seawater are not luminous because the autoinducer cannot accumulate, and luminescence develops only when conditions are favorable for the development of high population densities. This allows luminous bacteria to monitor their own population density.

Demonstration

We filled a long glass tube with a culture of *V. harveyi*, went to a dark room, adapted our eyes to the darkness and observed the luminescence. We turned the tube upside down several times to allow the oxygen present in the air bubble to diffuse into the medium.

Results

The enzyme luciferase is only active with oxygen because the *in vitro* light emitting reaction is coupled to aerobic oxidations according to the following scheme:



Appendix

Experimental protocol http://www.microeco.unizh.ch/uni/kurs/bio3_03/pdf/12biolum02.pdf
Chapters in BBOM 9th: 7.6 (Fig. 7.21), 13.11
Chapters in BBOM 10th: 8.7, 8.9, 12.12