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

Authors: Patrick Brunner s0170821@access.unizh.ch  
Angela Köllicker s0173033@access.unizh.ch  
Andrea Schiess s0171034@access.unizh.ch

Tutor: Munti Yuhana myuhana@botinst.unizh.ch

Objectives of the experiment

To understand gene transfer by conjugation in bacteria (triparental mating) and to learn about *ina* (ice nucleation active) gene expression in recipient bacteria by performing an ice nucleation assay.

To “create” a transconjugant bacterium from a phenotype able to glow in darkness which acquires the *ina* gene enabling the organism to catalyze ice formation. This can be achieved through a conjugation experiment. The experiment involves the recipient with a luminescent phenotype and a donor which provides the ice nucleation active gene (*ina*+) on plasmid pJL1703. The *ina*+ gene is responsible for catalyzing water crystallization. The plasmid is mobilizable, but it is not self-transferable. Therefore, the helper strain *E.coli* (pRK 2013) is required. This strain provides the *tra* operon.

In this experiment we used 2 strains of *Escherichia coli* and 1 strain of *Vibrio harveyi* with the following genotypes:

2) *E.coli* (pRK 2013): *tra+*, *ina−*, *Kanr*, *Amp*+, *lux−* (*helper/H*)
3) *V.harveyi*: *tra−*, *ina−*, *Kans*, *Ampr*, *lux+* (*recipient/R*)

Practical work:

For the first step we prepared bacterial cultures in liquid Luria Bertani (LB) medium. The cultures were incubated overnight.

![Diagram showing step-by-step procedure](image)

We filled four Eppendorf tubes with 1 ml of the **Recipient** culture, three tubes with 1ml of the **Donor** culture and another three tubes with 1 ml of the **Helper** culture. The tubes were centrifuged at 7000 rpm for 3 minutes to collect the cells.

As controls we took four Eppendorf tubes, each containing 750 µl LB medium and marked them R, R+D, D+H and R+H, respectively.
We divided the pellet-containing tubes into those four groups referring to those labels. Then we resuspended the pelleted-cells with 100 µl sterile LB medium. The cultures were incubated overnight at 30°C.

For the main experiment (triparental mating) we took one Eppendorf tube, containing 750 µl LB marked with R+D+H.

The pelleted-cells of the R tube were resuspended with 100 µl sterile LB liquid medium, mixed with D, and H cells, and put into the R+D+H Eppendorf tube.

All of R, R+D, D+H, R+H and R+D+H tubes were incubated overnight at 30°C.

Plating of the cells on Ampicillin, Kanamycin and LB medium.

The controls were plated undiluted onto LB +Amp + Kan medium.

The triparental mating (R+D+H) cells were diluted with 0.9ml LB medium also containing Ampicillin and Kanamycin. We plated 25 µl, 50 µl and 100 µl onto different LB + Amp + Kan media.

Testing the growing transconjugants for luminescent bacteria: We selected 10 individual colonies, picked and streaked them individually onto LB + Amp + Kan Agar medium and incubated overnight at 30°C. For assaying the ice nucleation capability, we scraped two loops full from the plate and resuspended the cells into a test tube containing 10 ml of sterile PBS (phosphate-buffered saline). We then put the assay tubes into the cooling bath (at -5°C) for 3 to 5 minutes.

The transconjugants were able to glow in darkness. After we diluted them in 10 ml phosphate-buffered saline solution and exposed them at -5°C in a cooling bath for 3 to 5 minutes the solution quickly freezes (= indication for the presence of ina).

**Discussion**

Why did it happen?

Let’s remember the genotypes of the two strains of *E. coli* and the one of *V. harveyi*:

*E.coli* (pJL 1703): tra⁺, ina⁺, Kan⁺, Amp⁺, lux⁻ (donor/D)

*E.coli* (pRK 2013): tra⁺, ina⁺, Kan⁺, Amp⁺, lux⁻ (helper/H)

*V.harveyi*: tra⁺, ina⁺, Kan⁺, Amp⁺, lux⁻ (recipient/R)

The plasmid of *E. coli* (pRK 2013) cannot be maintained in other bacteria since it belongs to the narrow-host-range-plasmid group. It is possible, however, to transfer it into another *E.coli* (pJL1703), because the recipient *E.coli* belong to the same species, and it carries the transfer operon (tra⁺). At the beginning of our experiment we prepared a few controls. First we just incubated PBS solutions containing *V. harveyi* cells in the cooling bath. As expected, the bacteria weren’t able to initiate freezing the solution at this temperature. The reason is that *Vibrio* does not have an ina⁺ gene.
For comparison we made 3 different diparental matings as controls:

recipient + donor (R + D),
donor + helper (D + H),
recipient + helper (R + H)

No growth was observed when we plated the controls undiluted onto LB (Luria Bertani medium) + Ampicillin + Kanamicin Agar plates. None of the D, H, or R has is resistant to both antibiotics. We propose the following explanations for these observations:

R + D: R is sensitive to Kanamicin (\textit{Kan}'), D is sensitive to Ampicillin (\textit{Amp}') and both are not able to transfer their plasmids to each other because they lack a transfer operon (\textit{tra}).

D + H: D is sensitive to Ampicillin (\textit{Amp}'), H is sensitive to Ampicillin (\textit{Amp}') too, so they are not able to grow on an Ampicillin-containing medium. They are able to exchange plasmids, however, since H contains transfer operon (\textit{tra}).

R + H: R is Kanamicin sensitive (\textit{Kan}'), H is Ampicillin sensitive (\textit{Amp}') and H is narrow host range. Although H carries a \textit{tra} operon neither R nor H can grow on media containing Kanamycin and Ampicillin.

With this knowledge we started our main experiment: The triparental mating. Bacterial mating (conjugation) is a process of genetic transfer that involves cell-to-cell contact. A conjugative plasmid uses the helper mechanism (\textit{tra} operon) to transfer a copy of its plasmid to a new host.

Transconjugant bacteria are able to grow on the LB+Kan+Amp medium, if they acquired resistance to Kanamycin in addition. If they are able to glow we know that they are \textit{V. harveyi} and if they are also able to quickly freeze the buffer solution, we have clear indication that they successfully acquired the \textit{ina} gene from the \textit{E.coli} donor.

Mating between \textit{E.coli} (donor) and \textit{E.coli} (helper): The donor receives the \textit{tra} operon from the helper. Transconjugant \textit{E.coli} (D+H) contain: \textit{tra}', \textit{ina}', \textit{Kan}', \textit{Amp}', \textit{lux} 

Besides carrying the \textit{tra} operon they will not glow and grow on LB+Kanamycin but not on LB+Ampicillin containing plates. Since the transconjugant donor is now \textit{tra}'', it is capable of conjugating with the final recipient.

Mating between the transconjugant \textit{E.coli} (\textit{tra}'') and \textit{V.harveyi}:

\textit{V.harveyi} transconjugants then contains: \textit{tra}'', \textit{ina}'', \textit{Kan}'', \textit{Amp}'', \textit{lux}'

\textit{V.harveyi} transconjugants (\textit{Kan}'', \textit{Amp}'') will grow on LB+Kan+Amp containing plates, while both, the original donor (\textit{Kan}'', \textit{Amp}''), the helper (\textit{Kan}'', \textit{Amp}'') and the \textit{E.coli} D+H transconjugant (\textit{Kan}'', \textit{Amp}'') will not be able to grow on LB+Kan+Amp containing media. Original recipient \textit{V.harveyi} (\textit{Kan}'', \textit{Amp}') will also not grow on LB+Kan+Amp plates.

This way the recipient gets \textit{tra}'', \textit{ina}'' and \textit{Kan}'' from the transconjugant donor (D+H). The findings of the triparental mating was a bacterial strain which is able to catalyze ice formation and to glow.

Appendix:


Reading Chapters 9.1, 9.5, 9.9 in BBOM 9th