Dynamics of Conjugation of *Enterococcus faecalis*

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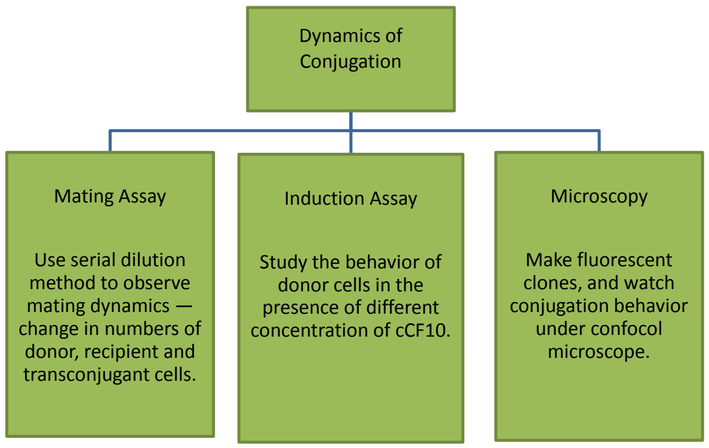
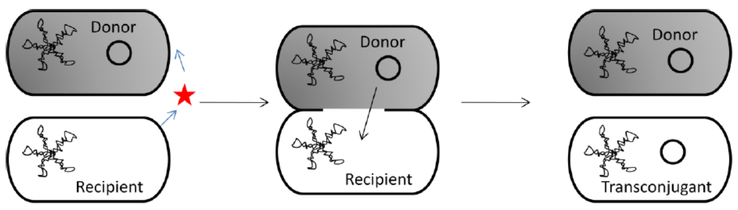
Components of Experiment



Background

Enterococcus faecalis is a gram positive bacterium normally residing in human intestine as a component of natural flora. It may act as a pathogen, however, for people with impaired immune system. *Enterococcus faecalis* is very efficient in acquiring resistance to antibiotics. One of its strains has been identified to be resistant to vancomycin—a drug that is traditionally used as the last resort. *Enterococcus faecalis* transfers resistance to antibiotics within and across species.



The transfer process of antibiotic resistance takes place through the transfer of plasmids—small circular DNA with genes encoded for antibiotic resistance. The most common and powerful way to transfer plasmids is conjugation. In our experiment, during the process of conjugation, cells without the desired plasmid (recipient cells) release signaling molecules (cCF10) into the environment, and induce the host cells (donor cells) with the desired plasmid. Once the donor cells are induced, they will conjugate with recipient cells and transfer the plasmid of interest—pCF10 which contains genes for antibiotic resistance, to the recipient cells. The initial recipient cells are thus converted to transconjugant cells, capable of acting as donor cells eventually.

In order to prevent self-induction by the cCF10 generated by donor cells themselves, donor cells produce an inhibiting signaling pheromone, iCF10. cCF10 and iCF10 competeswith each other, and only when cCF10 exists in a high enough concentration would the donor cells be induced.

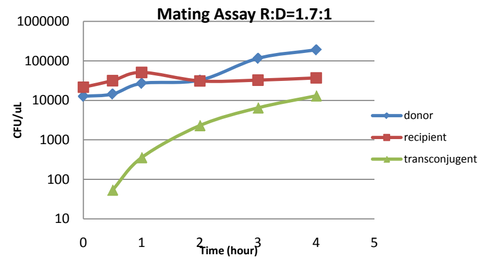
Mating Assay

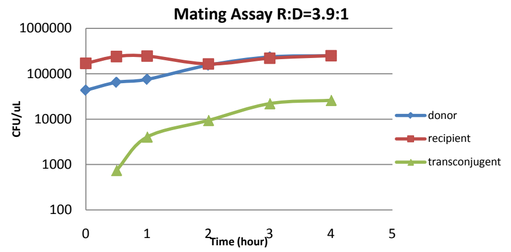
Protocol of Mating Assay

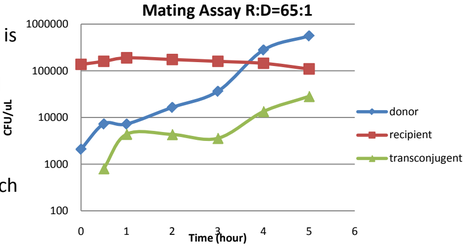
1. Donor and recipient cells were grown separately in M9 media at 37°C for 16 hours.
2. The cell cultures were diluted 1:5 and incubated for 1.5 hours
3. Donor and recipient cell cultures were mixed at different ratios.
4. Serial dilution method was used to get the information of the number of cells at different time points.
5. Donor and recipient cell colonies were counted after 24 hours and transconjugant after 48 hours.

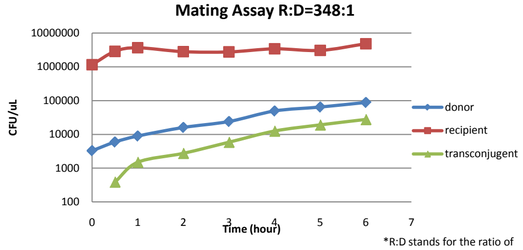
Interpretation of Results

One possible explanation for the wavy growth trend of recipient cells is that initially the recipient cells grew normally as they would in isolation. Meanwhile they released pheromone cCF10 which induced donor cells. The induced donor cells conjugated with recipient cells and thus transconjugant cells were engendered from initial recipient cells, i.e. some recipient cells were converted to transconjugant cells, which accounted for the decrease in population after 1 hour. With such decrease came the decrease in the concentration of cCF10 and conjugation, enabling the recipient cells to back up. And the circle started again.





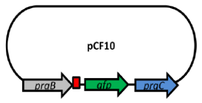




*R:D stands for the ratio of recipient to donor cells

Induction Assay

Fluorescent clones of *Enterococcus faecalis* were used in the induction assay. A gfp (green fluorescent protein) gene was incorporated into the target plasmid pCF10, such that once the cell was induced, the gfp gene would be expressed simultaneously and the cell would have green fluorescence. This fluorescence can be detected by FACSCalibur.



OFF→ON Assay

The object of this assay was to find out at what concentration of cCF10 would the pheromone be able to counterbalance the effect of iCF10 and induce the donor cells. In this assay, different concentration of cCF10 was added to the donor cell culture. The culture was then allowed to incubate for one hour for the signaling molecule to fully act. The result is presented below.

Concentration of cCF10 (ng/uL)	Status
0	OFF
0.01	OFF
0.1	OFF
1	ambiguous
10	ON
100	ON

Induction Time Assay

The object of this assay was to find out how long it took for the pheromone cCF10 to induce donor cells. In this assay, 10 ng/uL cCF10 was added to the donor cell culture. Samples were taken at different time points and analyzed to see if any fluorescence could be detected. The result indicated donor cells were inducted between 20 to 40 minutes after the pheromone was added.

Microscopy

Just like donor cells are genetically modified to express green fluorescence, recipient cells are modified to express red fluorescence. The gene MCherry was incorporated into the chromosome of recipient cells. The final goal of microscopy is to be able to observe green donor cells, red recipient cells, and green-red transconjugant cells simultaneously under the microscope. It will give us a vivid illustration of the time needed for induction, the duration of conjugation, the time required for transconjugant cells to act as donor cells. This assay is currently in progress.

Conclusions

1. The doubling time of donor cells of *Enterococcus faecalis* is approximately 50 minutes; the doubling time of recipient cells is approximately 55 minutes.
2. The donor cells of *Enterococcus faecalis* can be induced at the concentration between 1 and 10 ng/ul after cCF10 has been added for 20 to 40 minutes.
3. High concentration of cCF10 affects pellet formation.
4. MCherry expression of recipient cells was confirmed under microscopy.

References

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