FREQUENCY-DEPENDENT TRANSMISSION IN PLASMID CONJUGATION

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INTRODUCTION

Horizontal Gene Transfer (HGT) of plasmids and Integrative and Conjugative Elements (ICEs) mediated by conjugation is one of the most important mechanisms allowing the spreading of multi-drug resistances [1]. In order to plan effective measurements to stop the dissemination of antibiotic resistances, we need to understand the population dynamics of plasmid transfer. Conjugation is a biological process in which donor bacteria transfer a plasmid to recipient bacteria mediated by transfer proteins encoded in the conjugative element [2].

Previous works on conjugation rates on solid surfaces have provided information on primary transfer events [3]. Nevertheless, more data on transfer kinetics is needed to model mathematically the conjugation process, a basic requirement to predict plasmid dissemination in bacterial populations.

The common accepted model, known as "density-dependent model", assumes that spreading of plasmids has a transfer rate limited only by direct contact between a donor (infected) and a recipient (susceptible) cell, following a dynamic similar to that of flu epidemics [4].

In this work plasmids R388 and F were used as infecting agents to test the rate of plasmid transfer regarding the availability of susceptible *E. coli* recipients. Contrary to the original model, our results suggest that plasmid spreading does not follow a density-dependent dynamics, but a frequency-dependent one. This has important implications in the kinetics of plasmid dissemination and the mechanism of plasmid transfer.

MATERIALS AND METHODS

Mating experiments. They were carried out using the *E. coli* strain Bw27783 (Nx^R) as a donor strain of the conjugative plasmid (either R388 (Tp^R), or pOX38 (a Km^R derivative of the plasmid F)) and Bw27783 (Rif^R) as a recipient strain.
Different ratios of donor and recipient strains were used, estimated by OD_{600} measurements. Donor and recipients were mixed and plated onto LB-agar surfaces in a 24-well plate, at 37ºC, for periods ranging from 1-3h.
After mating, the cells were resuspended in PBS to avoid subsequent growth and dilutions were plated in appropiate antibiotics. Conjugation frequency was calculated as the proportion of transconjugants per donor or recipient cell.
Some mating experiments were carried out with serial dilutions from the original cultures of donor and recipient cells.
Charts indicate the average and confidence interval (significance level: 0.05) for n=9 replicates. They were calculated taking into account that rates of conjugation follow a log-Normal Distribution.

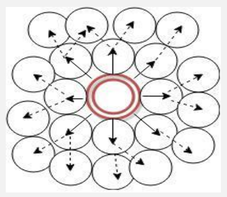
Mathematical model.
In epidemiology, the so-called "Force of Infection" (FOI or λ) is used to measure infectivity of a pathogen.
- r : rate of encounter per cell
- P_i: Probability of encounter being between donor and receptor.
- P_t: Probability of that encounter resulting in transmission.

$$\lambda = Pi \cdot Pt \cdot r$$

Growth on "infected" individuals will be the product of FOI and the number of susceptible individuals:

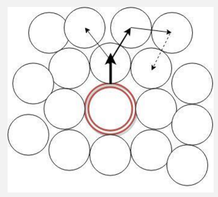
$$\frac{dT}{dt} = = \lambda \cdot N_{rec}$$

The Density-Dependent Transmission (DDT) model relies on the idea of transmission being only a matter of availability of receptors. On the other hand, the Frequency-Dependent Transmisison (FDT) model considers the phenomenon as a saturable transmission from the donor to the receptor:



Density-Dependent

$$\lambda = \left(c \cdot \frac{N}{V}\right) \cdot \left(\frac{D+T}{N}\right) \cdot \nu$$



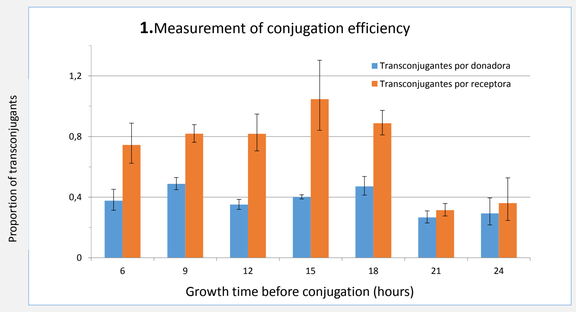
Frequency-Dependent

$$\lambda = c \cdot \left(\frac{D+T}{N}\right) \cdot \nu$$

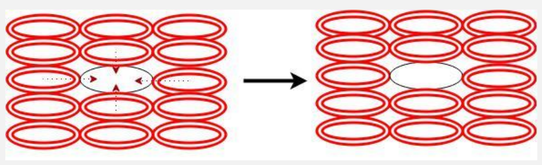
Serial dilution mating experiments in both liquid and solid media, were used to construct normalized concentration diagrams of population in order to test which of these two mechanisms is the one that R388 and F follows.

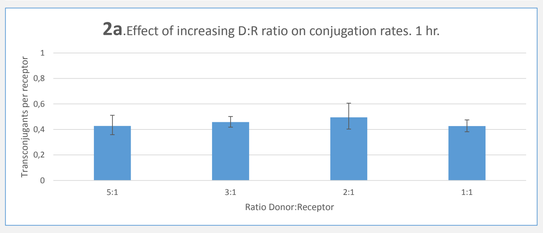
RESULTS

1.First rounds of experiments were developed with a 50% proportion of donors and receptors. Initial measurements showed noisy, but stable, results except for aged cultures :

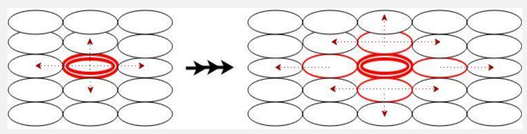


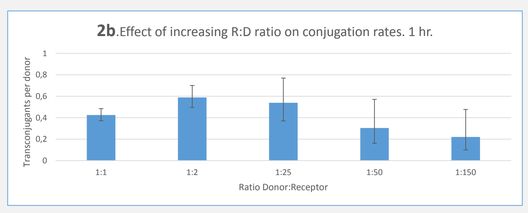
2a. Is there any limit imposed by recipients that could limit conjugation temporally?

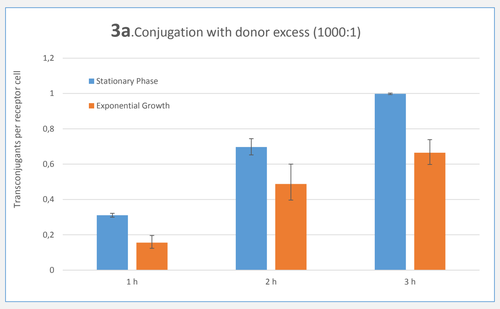


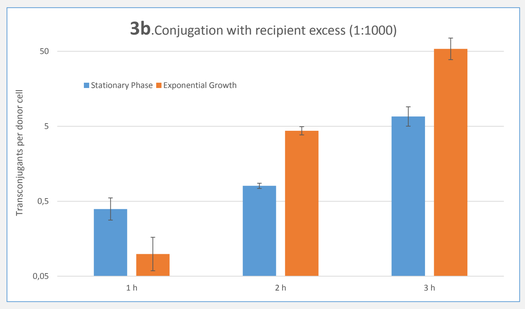


2b. Is there a limit to the number of conjugations a donor can carry out in one generation time?





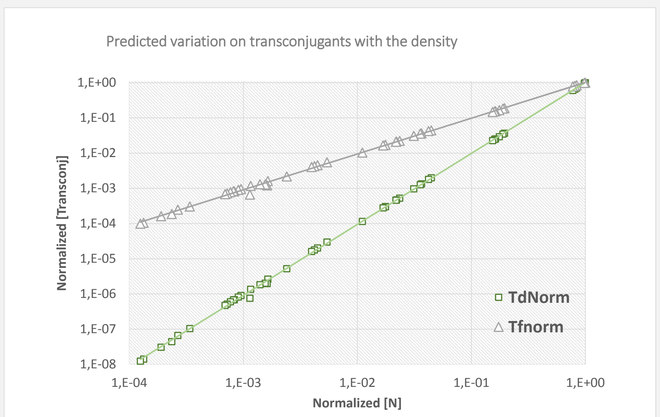


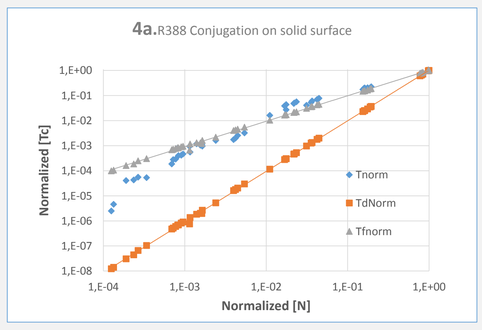


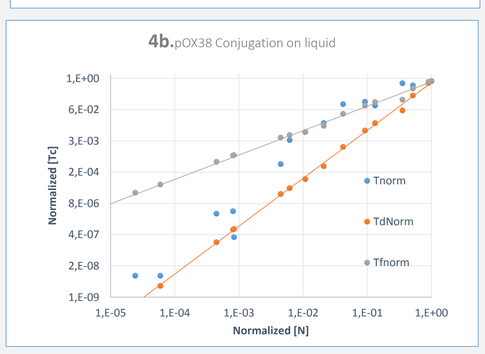
3a.- Longer mating times resulted in plasmid delivery to the 100% of recipient cells when donors outnumbered recipients by 1000 to 1. This suggest that **all recipient cells are susceptible to accept conjugation events**, the limit would not be in the number of donors or recipients but instead, it would be in the mating time and the time required for the cell to reach the optimal metabolic state. When donor and recipient populations were mated in exponential phase, conjugation rates decreased. Further studies on phisiological state and demographical effects have to be done.

3b.- Longer mating times with great excess of recipients (1:1000) shows increased transconjugant formation speed, probably due to secondary conjugation events with transconjugants acting as donor cells after a recovery time.

4.- Normalized [Tc] follows a Frequency-Dependent-Transmission fashion in almost all the range in solid (Fig.4a); transition to a density-dependent regime emerges at very low concentrations as recipient and donor cells become scarce. More pronounced deviations in the liquid experiment (Fig.4b) are attributable to 3D diffusion.







CONCLUSIONS:

- Conjugation is limited by both donors and recipients. That limit is measurable and does not depend on density for a broad range of population densities.
- We can conclude that conjugation follows Frequency-Dependent Dynamics; this is fundamental in order to develop population models on dissemination of antibiotic resistance genes.
- Further studies have to be carried out to determine more precisely conjugation rates with no secondary conjugation, to study conjugation rates *in vivo* and finally, demographic and metabolic effects have to be measured to be included in our model.

FUTURE PROSPECTS

- In the short term this study pave the way to: a) build mathematical models of plasmid dissemination and b) to test directed evolution experiments to improve conjugation rates.
- In a longer term we would like to create molecular tools to stop plasmid dissemination and generate bacterial computers based on plasmid conjugation.

Bibliography:
1) "The repertoire of ICE in prokaryotes underscores the unity, diversity and ubiquity of conjugation". *PLOS Genet*. 2011 Aug;7(8):, Guglielmini J, Quintais L, Garcillán-Barcia MP, de la Cruz F, Rocha EP.
2) "Towards an integrated model of bacterial conjugation". *FEMS Microbiol Rev*. 2015 Jan;39(1):81-95 Cabezón E, Ripoll-Rozada J, Peña A, de la Cruz F, Arechaga I.
3) "Determination of conjugation rates on solid surfaces". *Plasmid*. 2012 Mar;67(24)del Campo I, Ruiz R, Cuevas A, Revilla C, Vielva L, de la Cruz F.
4) "The kinetics of conjugative plasmid transmission: fit of a simple mass action model" *Plasmid*. 1979 Apr;2(2):247-60. Levin BR, Stewart FM, Rice VA.