A Spatial Model of Plasmid Conjugation Transfer on an Agar Surface

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Abstract: In our SDTC (space discrete and time continous) model we try to give a kind of abstractive 2.5D spatial description of bacterial plasmid conjugation transfer on the agar surface. We try to quantitatively trace each E.coli cell's state in our program. The correctness of our model is testified by our and others' experiment within acceptable error band. We also use the model to estimate the best proportion for conjugation efficiency between initial plasmid donor cells and recipient cells. Results show that with the total density of initial plasmid donor cells and recipient cells constant as 0.08 we get the best conjugation efficiency when the proportion 1.5.

Key words: Spatial model Cycle time trace Cell neighborhood Conjugation efficiency

1 Introduction of the SDTC Model

There are two main kinds of scheme to do the bacterial plasmid conjugation experiment: in well-mixed solution, or on solid culture such as agar surface. To spread the 'death gene' better in our 'toxic apple' project, we want to do some research on how to improve the conjugation efficiency under certain conditions. IGEM_Berkeley Team of IGEM 2005 have done some experiment on conjugation in solution and gave the best ratio between initial D(donor cells, similarly hereafter) and R(recipient cells) as 1:2. However, aimed to improve the conjugation efficiency, we consider the experiment and analysis of conjugation in solution are with these disadvantages:

1. The process of the experiment is difficult to be seen by an electron microscope.
2. The result is random in a certain degree due to the large space that cells could freely move in and the complex dynamics system of cells in the solution.
3. Mixing the solution is a necessary step to help D and R contact while it may also interrupt the conjugation itself.

Taking the reasons above into consideration, we decided to model for the scheme on solid culture. Due to the limited time in our experiment, we use some parameters from others' experiment and modeling and we also use the SDTC model to get the best ratio between initial D and R with the total number of initial plasmid donor cells and recipient cells constant by simulation.

In our SDTC model, we employ a lattice of 500×500 to describe each cell and each sites in simulation lattice area separately so we can trace their state separately at any time. We use a 'Cycle Time Trace' system to record each cell's doubling and conjugation state and ensure they update asynchronously so that the time in our system could be considered as continuous compared with the scale of cell doubling time or conjugation cycle time though any kind of simulation of time has to use a small period of time as simulation step length and we make no exception.

2 Description of the SDTC Model

2.1 Space system

In the SDTC Model, we set up a 500×500 lattice which contain 250000 sites. Bacteria and

nutriment can be at any site on the lattice. The bacteria cell is considered 1 um^2 that the total area of our view window is 0.25mm^2.

Furthermore, the lattice can be extended to more than 2 dimension. The thickness of lattice has three possible value: 0, 1, 2, that is to say, each site can hold mostly two kind of bacteria at the same time. In addition, there is no such 'up and down'difference inside one site in this 2.5D model. The thickness of nutriment is 0. The reasons why we set this model like this are as follows:

1. The model abstracts the thickness of bacteria into three levels, making the modeling and simulation able to be built and operated in several lattices, which greatly reduces the difficulty of modeling and the time of simulation.

2. Some three-dimensional properties are added to the SDTC model. For example, the filial generation comes out randomly around the parental generation. If the two layers of the neighborhood were taken over, the division will however be controlled. In a word, the SDTC model is a 2.5D model.

3. The model also largely simplifies the judgment of donor cell and receptor cell in conjugation (the conjugation occurs only when the donor cell and receptor cell are at the same site).

We introduce the conception of 'neighborhood' in our plane domain and divide it into 'bacteria neighborhood' and 'nutriment neighborhood'. As to any point, the bacteria of the bacterial neighborhood mean all the bacteria in the center of the 3×3 square area; similarly, the nutrition of the nutrient neighborhood refers to the nutrient in the center of the 7×7 square area. The detailed reasons of setting like this were discussed in Reference [2].

It is considered that bacteria can reproduce and transfer only within a certain distance due to the use of agar medium, but bacteria and nutrients cannot move. This is the fundamental difference between this model and the model in solution.

2.2 Bacterial and nutrient system

E. coli can be divided into three categories: D-donor, R-receptor, T-conjugative transfer receptor. Considering the bacterial horizontal conjugative transformation and reproduction, their speeds are related to the quantity of nutrients in the neighborhood, which is conformed to the beta function (a sigmoidal function). Specifically, there are two thresholds - the lower one C_1 and the upper one C_2. When the quantity of nutrients in the neighborhood $C<C_1$, reproduction will completely stop and the conjugative transformation will carry on at a very slow rate γ_1. When $C>C_1$, reproduction and conjugative transformation will do at a highest speed Ψ_2 and γ_2 respectively. Ψ_2 of the three bacteria D, R and T are different. When $C_2>C>C_1$, the speed of reproduction γ and the speed of conjugative transformation Ψ obey the distribution of C Linear within the minimum and maximum.

In the condition that there are nutrients in the nutritional neighborhood, D and R that are at the same site on the layers of different thicknesses begin conjugative transformation, while the division process always exists.

The repression has always been difficult in the modeling of conjugation. Most of the F plasmid is with repression. In our SDTC model, we deal the repression as the balance between the conjugation and division, or between the horizontal and vertical plasmid transfer . By introducing a penalty factor p and using our cycle time trace system, we are able to describe this balance. Once

a bacterium experience one type of plasmid transfer(horizontal or vertical), another type's cycle state of the site (which recorded by cycle time trace system) clear, as another type of plasmid transfer need to restart the preparation of energy and the bacterium itself. Besides, the cycle length of the second one change to p times of standard length ,which means the other kind of transfer need more preparation time.

We introduce a random factor τ to describe the plasmid loss. When D or T begins to split and multiply, their filial generation has the probability of τ lose the plasmid and become an R.

The filial generation that bacteria reproduce disperses randomly in the center of the 3×3 square area including them. But the offspring may 'die in the womb' when the number of bacteria around its site reaches the upper threshold due to the limited space in the medium.

Because of the use of agar medium, if the nutrient at a certain point is consumed, it is completely consumed and will not regenerate, nor be replenished by the surroundings.

Each reproduction consumes the nutrients in the nutrients neighborhood. If the nutrients in the neighborhood exhaust, the bacteria cannot reproduce. More precisely, they will firstly choose which distance-level of neighborhood nutrients to take in according to the proportion of the bacterial number and distance in the certain level(closer level has better chance), then, in the chosen level, which nutrient to choose will be determined by the random selection in direction. This algorithm has some advantages as follows:

1. The closer nutrients are more likely to be consumed.
2. Isotropic of nutrient consumption can be maintained.
3. The algorithm is simple.

2.3 Cycle time trace system

Set 40min as the standard time which receptor cell breed a generation take, which is to say Ψ_2 (R) =1.0. Ψ_2 (D), Ψ_2 (T), γ_2 are all the relative value to Ψ_2 (R). The rate of division and conjugation of bacteria at each point is decided by the quantity of nutriment in the neighborhood, making the update time of each point different. The details are as follows:

1. Initialize three cycles of tracking plane the same size as the simulation plane.
2. Two planes of two layers record the progress of bacteria's division cycle. Where there is no bacterium at the point of the plane, the progress of it is constant 0 .The third plane records the progress of conjugation cycle. The progress is not 0 only when there is a D and an R on the same site at the same time.
3. All the three planes need to be calculated at each step of the simulation. With the time of each simulation setted L minutes and r employed as the relative division rate or relative conjugation rate at that point of the plane. $r \times L/40+\xi$ will be added to each step and point according to rule 2, where ξ is a random factor. If the value of a certain point reaches or gets larger than 1 after a simulation, the value subtracts 1 and note the split /conjugation occurs once.

The advantage of this cycle time trace system is:

1. Being able to trace and record the progress of the division and conjugation at each point and plane in the cycle and make it convenient to improve SDTC to more complex models later.
2. SDTC model time can be considered as continuous. Because of the different relative division and conjugation rate of different cell, the update of each point on plane is

asynchronous. Actually, any simulation that operates with the time has to set a small step size. Once the step is tiny enough and the elements' update is asynchronous, we can say the time is continous.

3. Easy to deal with the program and simulation.

2.4 Initial state

D and R are randomly distributed of certain density in the two layer planes. The density of each plane can be controlled respectively. The nutriment filled the whole plane uniformly.

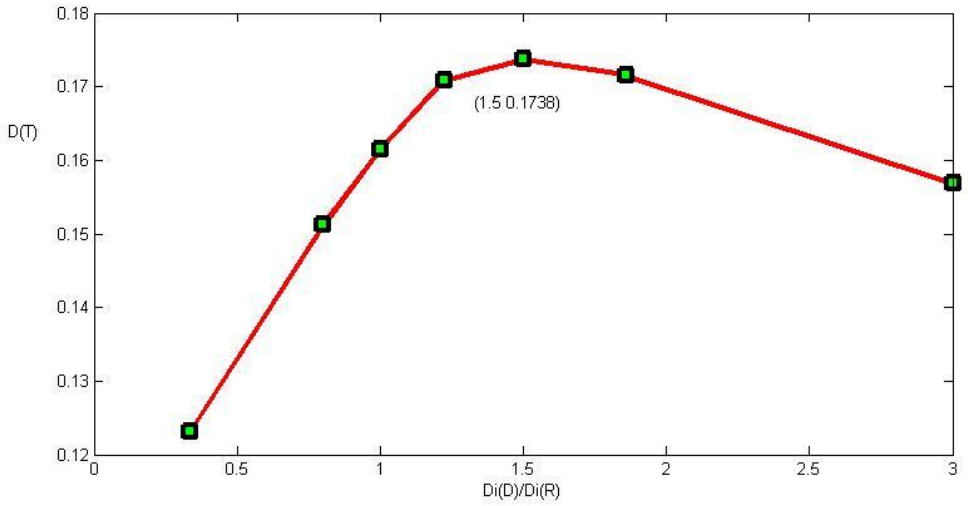
3 Model simulation and research on conjugative transfer efficiency

We use The SDCT model established above to simulate within the observation area of a lattice with its side length setted 500. Di (R) + Di (D) = n (Di (X) is the initial density, n is a predetermined value). We want to figure out the relationship of the ratio between Di (R) and Di (D) and final density D(T). .

We have the following description with the setting:

1. As the total number of the nutrition in the square region is limited, and competition occurs among conjugative transfer and reproduction nutrient. too high or too low initial density both cause conjugative transfer fewer. Therefore the initial density and must be limited within a certain value range.
2. Due to reproduction and conjugative transfer both breeding competition on nutrition, and we need to study the the ratio of D and R conjugative transfer efficiency, we should control the reproduction competitiveness, so then initial density is set to a fixed value.
3. the ratio of the optimum D and R may vary within a certain range with n. Here only consider one value of n.

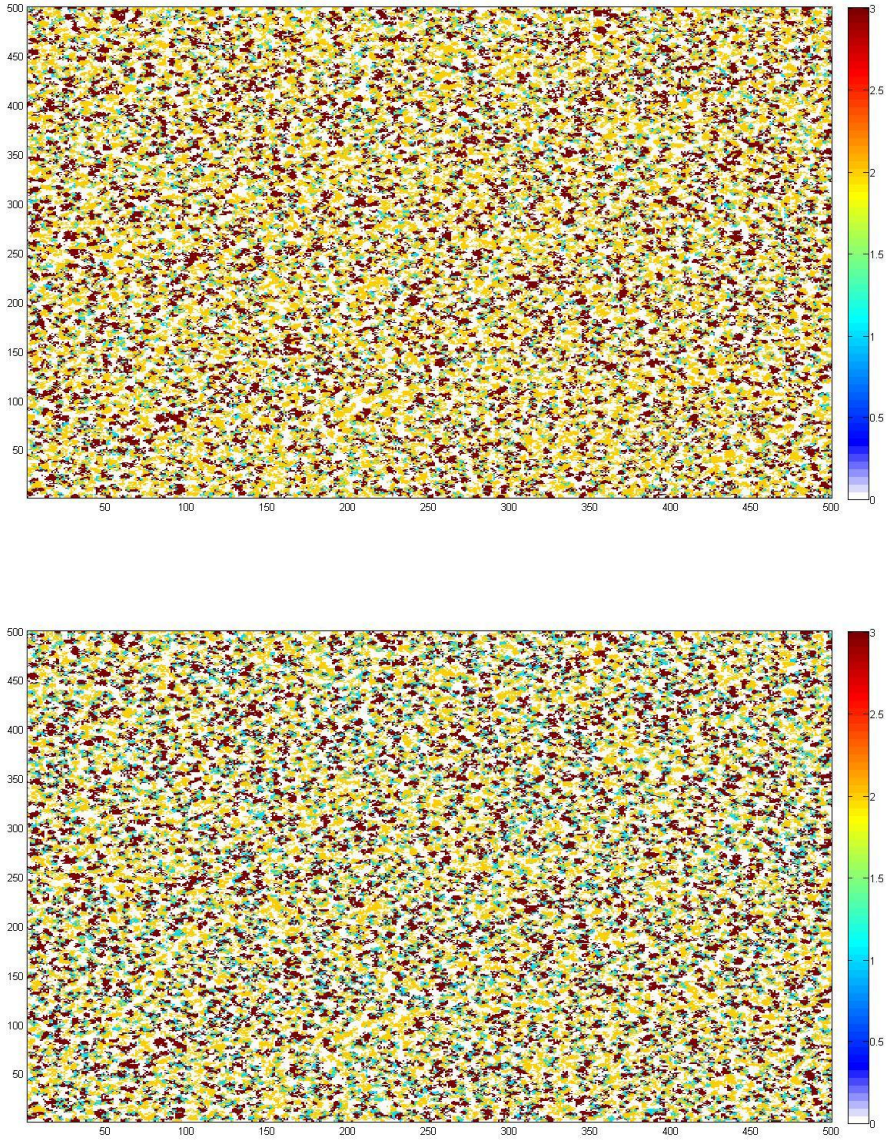
The simulation results are shown as below:



Wherein $C_2 = 4$, $C_1 = 1$, $\gamma_1 = 0$, $\gamma_2 = 6$, Ψ_2 (R) = 1.0, Ψ_2 (D) = Ψ_2 (T) = 0.9, $\tau = 0.005$, p = 1.0, n = 0.08.

Simulation step length is 1min. The maximum time limit of simulation is 30 hours. The simulation should be terminated when the total number of plane nutrition is less than the initial value multiplies 0.001.

As is shown in the picture, the best ratio of D and R is 3:2. The simulation is terminated as nutrients running out. The final state of the two layers of bacteria are shown below:



Red:R Yellow:D Blue:T

4 Shortcomings and improvements

1. Because of the limitation of experiment period and condition, some parameters in our simulation come from others' model and experiment. Since the experiment conditions are different, the parameters may be impertinent. We can calculate every parameter more carefully and improve the accuracy of model if we have more time and resources.

2. The model and simulation of the repression of conjugation has always been the difficulty of conjugation models, thus rarely can we find the related reference model and experiment. Set out with the conception of balance each other, we come up with a model of two plasmid transfer types which one can inhibit another one. But the correctness of this model still need more experiment to be testified.

3. Due to the limitation of condition, large-scale computers are not available. We could have

set a smaller simulation step length or improve the accuracy of simulation to acquire a better result.

5 Reference

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