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Mapping genes on an *Escherichia coli* chromosome

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Introduction

In order for a species to evolve, there must be variances in the genetic makeup to match outside factors, such as environment. These variances occur through the exchange of genetic material and recombination. Recombination results in gametes that differ from their parent cells. While this concept of genetic exchange is easy to understand in eukaryotes, it occurs differently in prokaryotes, which usually do not partake in sexual reproduction; rather, they reproduce by binary fission. The 3 ways in which bacteria accomplish gene transfer is through conjugation, transformation, and transduction. Transduction occurs when a bacterial virus acts as a carrier of DNA from one bacterial cell to another. Transformation is the process when a bacterial cell incorporates DNA from the environment, usually from a different, lysed cell. Conjugation, the focus of this experiment, occurs via physical contact, where one bacterial cell directly transfers DNA to another bacterial cell. Conjugation is the primary process through which antibiotic resistance spreads throughout bacteria, which makes it very important to study and comprehend (Coll et al. 2017).

Escherichia coli can act as an auxotroph, which means it is a mutant that requires an outside supply of select nutrients to survive and grow. In this experiment, 2 strains of *E. coli* were used separately and then mixed to test the theory of recombination by conjugation. In conjugation, one cell is considered F^+, meaning it has the fertility (F) factor, and the other is F^-. The F factor has an origin of replication and several genes that are required for conjugation (Pierce et al. 2014). The genes that are transferred from one cell to another are those that are present on the F factor itself. Thus, only F^+ cells and F^- cells can take place in conjugation. However, there is a third type of cell that can partake in conjugation, and this is a high frequency recombinant (Hfr) cell. Hfr cells have the F factor as integrated into the bacterial chromosome by homologous recombination (Pierce et al. 2014). Therefore, Hfr cells behave as F^+ cells, only they also transfer a copy of the bacterial chromosome to the new cell. For this experiment, one strain was Hfr, CSH121. The other strain was F^-, CSH125. CSH121 is Str^S, Leu^+, His^+, Arg^+, Ade^+, Trp^+, and Tet^R. CSH125 is Str^R, Leu^-, His^-, Arg^-, Ade^-, Trp^-, and Tet^S. The 2 strains were mixed and plated on Petri dishes that contained streptomycin and no leucine and their growth was monitored to test for conjugation.

When conjugation occurs between an Hfr strain and an F^- strain, a gradient of transmission occurs. The gradient of transmission suggests that the closer a gene is to the origin of replication,

the higher chance it has of being transferred to the new cell. This determination of the location of genes on a chromosome is called linkage mapping, which was developed with the real chromosomal structure in mind (Griffiths et al. 2000). In this experiment, the linkage map for an *E. coli* chromosome was derived following the percentage of inheritance, or the gradient of transmission.

The purpose of this experiment was to study conjugation between two strains of bacteria in a physical setting and to map the gene markers on the *E. coli* chromosome. The hypothesis for this experiment was that the CSH121 and CSH125 strains of *E. coli* will not have any growth on the original plates containing streptomycin and no leucine. Furthermore, it is hypothesized that each gene will transfer over during conjugation.

Materials and Methods

Week 1:

To begin, 3 test tubes were obtained and labeled as follows: a) CSH121/CSH125 for conjugation, b) CSH121 (control), and c) CSH125 (control). Then, the mouth of the CSH121 test tube was run through the flame of a Bunsen burner for sterilization. With a P2000 pipette, a sample of CSH121 (200 microliters) was transferred to the CSH121 test tube. The mouth of the tube was once again flamed, and a lid was placed on the tube. With a clean pipette tip, CSH121 (200 microliters) was added to the flamed CSH121/CSH125 test tube. The mouth of this tube was also flamed before placing the lid on it. The sterilization techniques were continued as CSH125 (200 microliters) was added to both the CSH125 test tube and the CSH121/CSH125 test tube. All 3 tubes were placed in the 37˚C water bath for 1 hour to incubate.

Using the same sterile technique that was used earlier, LB broth (2 mL) was added to each test tube. The tubes were then placed into a 37˚C shaker water bath for 45 minutes. While the tubes were incubating, 3 Petri dishes containing streptomycin and no leucine were labeled with experimenter name, section number, date, and then its respective serial dilution number: 1:100, 1:1,000, and 1:10,000. The Petri dishes for the controls, CSH121 and CSH125, were also labeled as such without the serial dilution number. Then, 4 microcentrifuge tubes were labeled as 1:10, 1:100, 1:1,000, and 1:10,000. Minimal media (90 microliters) was added to each microcentrifuge tube. 4 more microcentrifuge tubes were labeled as CSH121 1:10, CSH121 1:100, CSH125 1:10, and CSH125 1:100. Minimal media (90 microliters) was also added to these 4 microcentrifuge tubes.

When the incubation period was over, using the same sterile technique, CSH121/CSH125 (10 microliters) was added to the microcentrifuge tube labeled as 1:10. The tube was mixed by flicking the side. Then, a serial dilution was carried out through the 1:10,000 microcentrifuge tube, using 10 microliters of solution from the previous microcentrifuge tube. This same process was done for the controls.

Using a micropipette, the contents of the 1:100 microcentrifuge tube were transferred to the Petri dish labeled 1:100. Then, with a flamed inoculating loop, the bacteria were spread throughout the dish evenly. This was done for the 1:1,000 solution and the 1:10,000 solution, flaming the loop between each microcentrifuge tube and using a new micropipette tube each time. Then, the 1:100 dilutions of the controls were plated onto their respective Petri dishes. The dishes were incubated at 37˚C for 24-48 hours and then placed into a refrigerator until the next lab period.

Week 2:

The Petri dishes from the previous week were obtained and observed. Then, the dish labeled as CSH121/CSH125 1:100 was chosen for the source of colonies. On the lab bench there were 7 new Petri dishes, labeled as such: Leu^-, His^-, Arg^-, Ade^-, Trp^-, Tet, and Leu^-. These dishes were each placed over a grid of 50 squares. With a sterile toothpick from the lab bench, a single colony from the CSH121/CSH125 1:100 dish was picked up. The toothpick was used to pierce the agar at the number 1 square on the first grid under the Leu^- dish. With the same toothpick and bacteria colony, the same location (1) was pierced on each sequential dish, ending with the second Leu^- dish. Ending on the second Leu^- dish establishes a control, to ensure that sufficient bacteria was procured to be carried through all 7 dishes. This same procedure was done until each square on the grid, all 50 positions, were used. The Petri dishes were labeled with the experimenter name, section number, and date. These dishes were then placed in a 37˚C incubator for 24-48 hours. Then, they were placed in a refrigerator until the next lab period.

Week 3:

The 7 Petri dishes were obtained from the TA bench, and they were observed. The number of colonies on each dish were recorded, including the lab partner's numbers in the total so that way the total was an even 100 count. Any colonies that grew on the first Leu^- dish but not the second were discarded and not used in the total number.

Results

The relative amount of growth on each Petri dish from the first week of this experiment was observed. The dish containing the 1:100 dilution of CSH121/CSH125 had the most growth, followed by the dish containing the 1:1,000 dilution, with the 1:10,000 dilution containing the least amount of colonies. Both the control dishes, CSH121 and CSH125, had no bacteria growth. When the 7 Petri dishes from the second week of this experiment were observed, the number of colonies per dish were counted. The lab partner's 7 Petri dishes were also included in the total calculations. This data can be seen in Table 1.

Table 1: The number of colonies of CSH121/CSH125 found on each gene marker plate. *This table lists the number of colonies shown on each dish containing or lacking the respective gene marker. The data was also used to calculate the percent linkage, the log percent, and then the map position of each gene marker on the E. coli Hfr CSH121 strain.*

Marker	**# of colonies**	**% linkage**	**Log(%)**	**Map position (min.)**
Leu^-	100	100 %	2	2
His^-	8	8 %	0.90	44
Arg^-	0	0 %	-	78
Ade^-	66	66 %	1.82	8.9
Trp^-	42	42 %	1.62	16.5
Tet	80	80 %	1.90	5.8

Using the known map positions of Leu^- and His^-, a graph portraying the percentage of inheritance (log %) against the map position was created, shown in Figure 1.

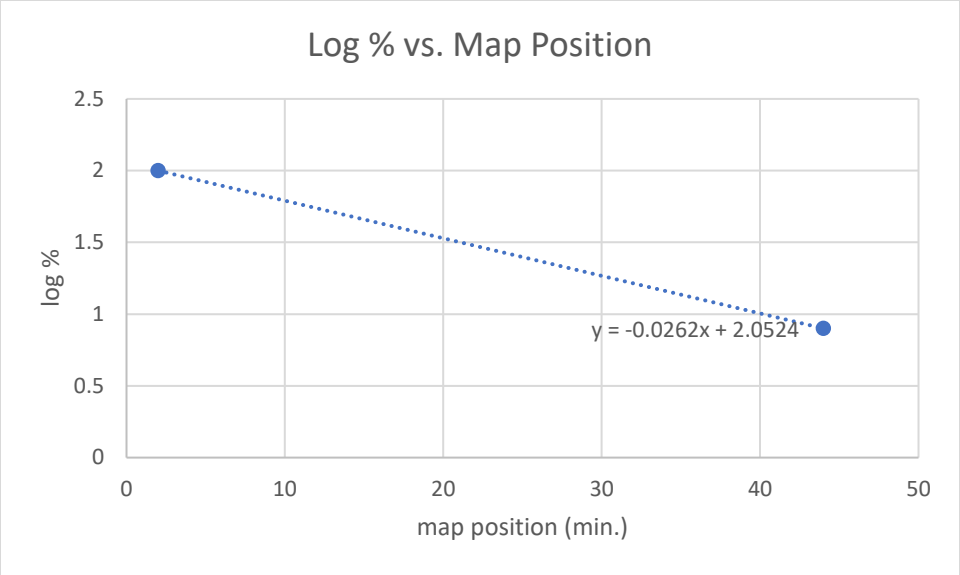

Figure 1 *is the graph of log % versus map position of leucine and histidine on the E. coli Hfr CSH121 chromosome.*

The line of best fit between those 2 points was used to calculate the map position of the rest of the gene markers, also seen in Table 1. Then, using the map position of each marker, a linkage map of the Hfr strain CSH121 was drawn up, shown in Figure 2

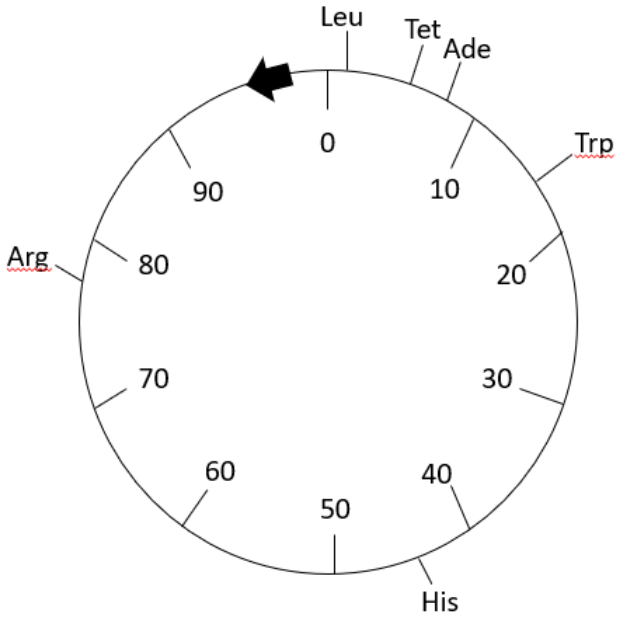

Figure 2: The linkage map for the CSH125 Hfr chromosome. *This is the potential linkage map for each gene on the E. coli CSH125 Hfr chromosome with its markers located in "minutes" from the origin of replication.*

Discussion

Based on the data listed in Table 1 and the linkage map shown in Figure 2, it can be concluded that Leu, Tet, Ade, and Trp are closest to the origin of replication, as they were the most numerous. No bacteria colonies grew in the Petri dish labeled as Arg^-, which indicates that Arg is quite far from the origin of replication, almost to the point where it could be considered genetically unlinked to the rest of the genes. Also based on the comparison of the dilution CSH121/CSH125 plates to the control plates from week 1, it can be concluded that conjugation did take place, and it the 2 strains were actually very successful in creating the Hfr strain of *E. coli.*

The purpose of the controls in this experiment was to establish a way to compare the growth and survival of each *E. coli* strain because neither strain could survive on its own in the environment they were put into. Furthermore, the second Leu^- dish in part 2 of this experiment acted as the control, because if there were not enough bacteria to be transferred all the way through, then it was understood that the data for those grid squares was inconclusive.

It was necessary to be able to obtain single colonies in this experiment because each colony of bacteria is genetically unique. And, in each individual colony, all the bacteria are genetically identical. Thus, obtaining single colonies ensured that all the genes were the same for each colony selected, which avoids errors and false conclusions.

Based on the data in Table 1 and the linkage map shown in Figure 2, the approximate locations in minutes of Arg, Ade, Trp, and Tet can be determined. Arg is located at around 78 minutes, Ade around 8.9 minutes, Trp around 16.5 minutes, and Tet around 5.8 minutes. Thus, Tet is closest to the origin of replication, after Leu, and Arg is the farthest.

If another Hfr strain, CSH119, had all the same markers as CSH121 and was used in an experiment to determine the approximate location and orientation of the origin in CSH119, the procedure would be very similar to the procedure used in this experiment. Instead of using CSH121, CSH119 would be mixed with CSH125. CSH119 would act as the F^+ cell and CSH125

would continue to act as the F^- cell. The experiment could then follow the exact method used here.

In conclusion, the hypothesis for Week 1, that only the plates with CSH121/CSH125 would show growth, was correct. Furthermore, the hypothesis that each gene would be transferred over was not supported, as Arg did not transfer over at all. However, this can be attributed to its far distance from the origin of replication on the CSH121 bacterial chromosome, as the farther a gene is from the origin, the less likely it is to be transferred. In general, the results from this experiment enforce the concept of conjugation and further support the idea that, in order to survive, a species must adapt to the environment it is exposed to, or else it will be extinguished.

References

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