Cantaurus, Vol. 18, 50-52, May 2010 © McPherson College Division of Science and Technology

The Effects of Temperature on the Rate of Conjugation in *Escherichia coli.*

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ABSTRACT

The effects of temperature on the rate of conjugation were studied in *E. coli* using plasmids which code for resistance to Ampicillin and Kanamycin. Cultures were grown and mated at 25°C and 37°C to create a wide temperature range to maximize observable differences in transfer frequency. Results were inconclusive due to difficulties in obtaining transfer frequencies. However, results of the 25°C test supported earlier reports by Al-Masaudi et.al. that transfer frequency remains high even at room temperature.

Keywords: *Antibiotic resistance, bacterial conjugation, E. coli, temperature, transfer frequency*.

INTRODUCTION

Bacterial conjugation is a topic of increasing interest in the scientific world due to its contributions to the interspecies transfer of genes coding for antibacterial resistance (Garcillan-Barcia, 2007). Gaining an understanding of this process will prove useful in assessing the impact conjugation might have in coming years.

Conjugation is the transfer of non-essential genetic information from one bacterium to another. This is a promiscuous process which requires that a donor cell (F+) produce a sex pilus and physically connect to a recipient cell (F-). The transferred genetic material exists in the form of DNA plasmids—circular pieces of non-essential, non-genomic DNA. Once inside the recipient cell, the plasmid may then incorporate itself into the recipient cell's genome. Along with the circular plasmid, the donor cell also transfers an F factor, which is required to initiate conjugation. The recipient cell becomes an F+, and can continue to spread antibiotic resistance. (Brooker, 2009).

It is evident that conjugation opens up numerous avenues for the lateral genetic transfer of antibacterial resistance among hundreds of strains of harmful bacteria, rendering antibiotics virtually useless (Llosa et al. 2002). This is a problem wherever antibiotics are being applied e.g. human antibiotics or the food industry. How then, can the spread of antibacterial resistance be slowed or arrested?

Temperature is used in the food industry to control and prevent the growth of unwanted bacteria on/in food products, such as meat and dairy products. It would be logical to hypothesize that temperature also has an effect on microbial processes other than growth. Incubating an organism at a temperature lower than its optimum should generate a lower incidence of conjugation, given that the organism is under stress.

Due to the broad applications of information regarding bacterial conjugation, my goal is to determine the effects of different incubation temperatures on the rate of conjugation in *Escherichia coli.*

MATERIALS AND METHODS

Model Organisms

Two live slant cultures of *Escherichia coli* were obtained from Carolina Biological Supply. The first, *E. coli* pAmp, contains the resistance plasmid for Ampicillin (Carolina item#211540). The second, *E. coli* pKan, contains the resistance plasmid for Kanamycin (Carolina item#211550).

Mating

The two *E. coli* cultures were raised in separate 150mL Erlenmeyer flasks of 50mL of Nutrient Broth (NB) sealed with tin foil. The cultures were allowed to grow for 24 hours at 37°C for the 37° Celsius test or 25°C for the 25° Celsius test. A flamed wire loop was used to inoculate the flasks from cultures kept in a refrigerator at 4C. After the 24 hour growth period, the *E. coli* pAmp culture was poured into the *E. coli* pKan culture flask and swirled vigorously by hand for five seconds. This process was performed in a biosafety cabinet to prevent contamination. The conjugative mixture was then placed in either the 37°C or 25°C incubator, depending on the current test conditions, for one hour to allow conjugation to take place.

Antibiotic Solutions

Ampicillin dry powder (Carolina Biological Supply) and Kanamycin dry powder (Carolina Biological Supply) were obtained for use in the selective media. I prepared a 50mL Kanamycin solution at 10mg/mL, and a 50mL Ampicillin solution at 10mg/mL.

Plating and Selective Media

I used autoclaved glass Pyrex beads to plate my controls and samples on 100x15mm Petri plates. I

poured the beads from a sterile beaker onto the plates. After 400uL of the culture to be plated was added using a micropipetter, I slid the plate back and forth on a benchtop in a "figure eight" pattern until the broth covered the entire surface of the agar. I then emptied the beads into a waste beaker by tilting the plate to the side and opening the plate in a "clamshell" fashion. Approximately six beads were sufficient to achieve an even spread of the inoculated solutions across the surface of the agar.

I used three different types of selective agar. Nutrient Agar (NA) with 2mL Ampicillin solution per 100mL of agar, NA with 0.15mL Kanamycin solution per 100mL of Agar, and NA with both Kanamycin and Ampicillin using the aforementioned concentrations.

Controls
Table 1 illustrates the controls and the factors they control for.

Table 1. Controls for the antibiotics used in the experiment. G=Growth Expected, NG=No Growth Expected

Bacterial Strain	NA	NA+Amp	NA+Kan	NA+Kan/Amp
E.coli/pAmp	G	G	NG	NG
E.coli/pKan	G	NG	G	NG
Conj. mix	G	G	G	G *Samples

Serial Dilutions
In order to obtain a countable number of transconjugant colonies on sample plates, I performed a serial dilution of the conjugative mixture prior to plating on selective media. The conjugative mix was diluted to concentrations ranging from 10^{-1} to 10^{-6} in order to find a suitable dilution.

I performed a second serial dilution following the necessity for a new sample concentration. I diluted to concentrations of 1:25, 1:75, 1:100, and 1:150

Technique Assessment
In order to assess the quality of my technique, I diluted a culture of *E. coli* pAmp grown for 24 hours to a concentration of 10^{-6}. I then plated ten samples of 400µl on NA to assess the variation between the ten samples.

RESULTS

37° Celcius Test
At 37C, I was unable to find a concentration of the conjugative mixture which produced a countable number of colonies on my sample plates. At a concentration ratio of 1:25, the plates produced a number of colonies too numerous to count (Figure 1). However, at concentrations of 1:75, 1:100, and 1:150, there were only between one and ten colonies per plate.

Given that these observations did not represent the linear trend expected with a serial dilution, I suspected that there may be some errors in my technique that was affecting my ability to achieve repeatable results. Therefore, I conducted an assessment of the accuracy of my technique.

The test of ten samples produced a mean colony count of 56.4 colonies per plate with a range of 27 colonies and a standard deviation of 8.9 colonies.

25° Celcius Test
At 25° C, *E. coli* are expected to produce virtually no F pili (Novotny, Lavin, 1971). However, upon completing the test, my samples showed growth covering the entire surface area of each of the ten sample plates. It is important to note that the *E. coli* used to inoculate the *E. coli* pAmp and *E. coli* pKan cultures used in this test came from cultures kept in a 4°C refrigerator and were immediately inoculated into 25°C nutrient broth. Therefore, the bacteria were never allowed to reach a temperature exceeding 25°C.

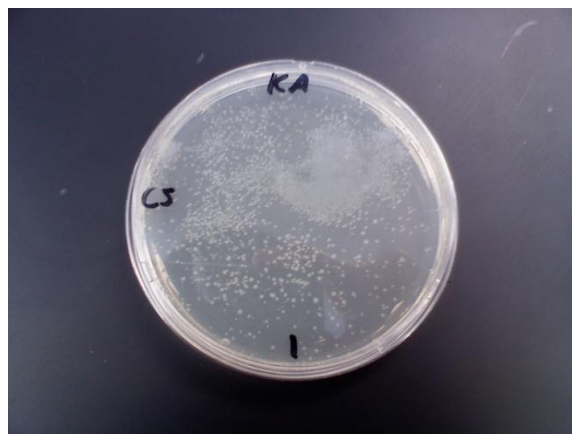

Figure 1. Transconjugant colonies at 1:25 dilution on NA with Ampicillin and Kanamycin.

DISCUSSION

The results and findings of this experiment are inconclusive as a result of the unanticipated difficulties in obtaining countable numbers of colonies on sample plates.

However, the growth of *E. coli* on selective media in the 25°C test does contradict earlier findings which stated that at 25°C, F pili are not being produced by *E. coli* (Novotny and Lavin, 1971).

Evidence of high frequency genetic transfer at 25°C does support similar findings which stated that the incubation temperature was not critical in determining the frequency of plasmid transfer in *Staphylococcus aureus*. Al-Masaudi, et. al. studied

factors affecting conjugative transfer in *Staphylococcus aureus*, with temperature as one of the factors under examination. They found that between 25°C and 42°C, there was no significant difference in the maximum transfer frequency in *S. aureus.* At 18°C, transfer frequency was significantly reduced but still measurable; at 10°C, conjugative transfer was undetectable (Al-Masaudi, et.al., 1990).

These findings are consistent with the results of this study. Although a transfer frequency could not be obtained, there appeared to be little or no effect on the transfer frequency from 37°C to 25°C, as indicated by the abundant growth of transconjugants on selective media.

Further investigation into the effect of temperature on the rate and frequency of conjugation in *E. coli* would need to be conducted utilizing a different approach in order to obtain significant data.

Determination of the consistency of the rate of conjugation among separate mating events would also be desirable. If the degree of variation in the rate of conjugation at a given temperature is too great, it may invalidate the significance of any data obtained regarding the effect of temperature on the rate of conjugation.

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