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Conjugation: A Preliminary Study of Recombinant DNA Technology (RDT)

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Abstract

The present study investigates bacterial conjugation as a key mechanism of horizontal gene transfer and its relevance to Recombinant DNA Technology (RDT). Conjugation enables the transfer of plasmids between bacterial cells through direct contact, mediated by the *tra* gene and the Type IV secretion system. Using *Escherichia coli* as a model organism, this experiment demonstrates the transfer of tetracycline resistance from an F^+ donor to an F^- recipient strain. The process was carried out using a specialized GeNie conjugation kit, followed by antibiotic screening on Luria Bertani agar media supplemented with tetracycline and streptomycin. Growth observed on dual antibiotic plates confirmed successful plasmid-mediated transfer of the resistance gene, validating the occurrence of conjugation. The findings are consistent with earlier reports describing conjugative plasmid transfer as a major contributor to antibiotic resistance dissemination. Beyond its biological significance, the study highlights the application of conjugation in genetic engineering and molecular cloning, where it serves as a foundational process for DNA manipulation. Overall, this research provides both theoretical and experimental understanding of bacterial conjugation, emphasizing its role in evolution, antibiotic resistance spread, and biotechnological applications.

Keywords: Bacterial conjugation, *Escherichia coli*, horizontal gene transfer, antibiotic resistance, recombinant DNA technology, plasmid.

Introduction:

Bacteria are the prokaryotic unicellular organism with no defined nucleus or other aspect as compared to eukaryotic. In bacteria the reproduction takes place asexually and which doesn't follow cell cycle. Bacteria are abundant in the surrounding environment and evolve throughout the time, for example become resistant, sensitive or in any other aspect. So the question arise how does this resistance develop? Bacteria had already evolved mechanisms to survive these natural antibiotics. These resistance genes evolved over millions of years through Random mutations in their DNA (Davies, J., & Davies, D. 2010). Horizontal gene transfer (bacteria can swap genes, including resistance genes, with each other). Conjugation is a type of horizontal gene transfer, here the plasmid (extra chromosomal DNA) get transferred from the F^+ (Donor) to F^- (Recipient) by cell to cell contact, the formation of intercytoplasmic bridge through sex pilus (Cabezón *et al*., 2015; Ilangovan *et al*., 2015). Conjugation was first Discovered and demonstrated by Lederberg and Tatum in 1946 (Lederberg & Tatum, 1946; Clark & Adelberg, 1962)

This study is focused on completely studying the process of conjugation practically and theoretically in

E. coli (gram negative bacteria). The theoretical explanation of conjugation is transfer the extra chromosomal DNA (plasmid), the gene which is responsible for the transfer of the plasmid DNA is 'tra gene located in the conjugative plasmid. However if we'll see the whole process of conjugation by plasmid encoding protein containing 'tra' gene, starting by formation of a bridge between the F^+ donor to F^- recipient, later the nick is formed at *ori* site on the one strand which is transferred to recipient and the other un-nicked strand is left in donor. While the transfer the single strand plasmid DNA is the replicate in both donor and recipient. This entire process need many protein produced by plasmid DNA (Willetts & Wilkins, 1984; Llosa *et al*., 2002; Phornphisutthimas *et al*., 2007).

Material and methodology:

Day 1 the sterilization of every autoclavable glassware was done, later the Media preparation was done and then the calculated antibiotics by concentration are dissolved in autoclaved media. Then plating is done. With inoculation of sterilized culture form lyophilized vials given in the kit and in Day 2 the culture is prepared for further used by the bacteria grown on the plate. Day 3 the OD (Optical Density) was taken till the reading appears to 0.8-0.9 then on day 4 the conjugation was done (mixing of both Donor and Recipient) and incubated for overnight. And on Day 5 the screening was done to understand the conjugation. Preparation of LB Broth/Agar (1 litre): Dissolve 25 g of LB Broth in 800 ml of distilled water. Adjust the pH to 7.0 with SN NaOH (if necessary) and make up the volume to 1000 ml. Sterilize by autoclaving. For LB agar, add 1.5% agar and autoclave. Preparation of Antibiotic Tetracycline Dissolve 45 mg of Tetracycline supplied in 1.5 ml of 70% ethanol. Vortex if necessary. This gives a stock concentration of 30 mg/ml. Cover the vial with aluminum foil and store at 4°C. Use the antibiotic solution within 2 weeks. Add this antibiotic solution to LB medium at a concentration of 30 µg/ml. Streptomycin; Dissolve 150 mg of Streptomycin supplied in 1.5 ml of sterile water. Vortex if necessary. This gives a stock concentration of 100 mg/ml. Cover the vial with aluminum foil and store at 4°C. Use the antibiotic solution within 2 weeks. Add this antibiotic solution to LB medium at concentration of 100 µg/ml (Phornphisutthimas *et al*., 2007; Alalam *et al*., 2021). The procedure is also prepared by studying the manual of the GeNie conjugation kit as well as the research papers and as followed:

Day 1: Revival of Parental Strains.

The F+ donor strain lyophilized vial is braked open and suspended with the 0.1 ml of sterile LB broth. Similarly the F^- recipient strain is open and suspended with 0.1 ml of sterile broth. Then carefully the 25 µl of Donor strain is inoculated in duplicate on the sterile media plate with the tetracycline antibiotic at concentration of 30 µg/ml and recipient strain on streptomycin with concentration of 100 µg/ml and remaining 50 µg/ml of both donor and recipient strain is inoculated in broth with respective antibiotics and incubate it at 37°C recipient strain is inoculated in broth with respective antibiotics and incubate it at 37°C for overnight.

Day 2: Resuspension of the Parental strain

Picking the single colony separately from both donor and recipient strain and inoculate in the 6 ml LB broth containing the antibiotics tetracycline and streptomycin respectively in two different tubes. Then these test tubes is again incubated it at 37°C for overnight.

Day 3: The OD reading

Pipetting the 1 ml of overnight incubated donor culture from day 2 in the 25 ml LB broth containing Tetracycline of 30 µg/ml concentration. Similarly incubate the 3 ml of recipient culture in 25ml LB broth containing the streptomycin of 100 µg/ml concentration and incubates it in a shaker incubator at 37°C till the donor culture OD reaches to 0.8-0.9 at A_{600}, (E. coli generation time is roughly around 20-30 minutes, so if the reading of donor culture after inoculation is 0.1 then OD A_{600} of 01 to 08 means roughly 3 doublings since log:(0.8/0.1) 3 then after 3 doubling-30 minutes 90 minutes and if some variations occurs we roughly take it as presumption of around 2.5-3 hours).

Day 4: Conjugation:

After the OD of donor culture reaches to around 0.8-0.9 at A600 then pipette the 0.2 ml of both donor and recipient cultures in sterilized cotton plugged test tube and mix it for a few seconds and incubate in incubator at 37°C for 1 hour 30 minute. Simultaneously take 0.2 ml of both donor and recipient culture (from day 3) in separate test tube and incubate at 37°C for 1 hour 30 minute. After the incubation add 2 ml of sterile LB broth in all three sample, the conjugated one, donor one and recipient one and again incubate at 37°C for 1 hour 30 minute. Now pipette out 0.1 ml of each sample in petri plate according to the table 1 given below.

	F^+ strain	**F^- recipient strain**	**Conjugated Sample**
LB + streptomycin	0.1 ml	0.1 ml	0.1 ml
LB + Tetracycline	0.1 ml	0.1 ml	0.1 ml
LB + streptomycin + tetracycline.	0.1 ml	0.1 ml	0.1 ml

Table 1: Plating of sample for final screening

Now after the plating of sample according to the table 1, incubate the all plates at 37°C for overnight and record the observation.

Observation:

	F^+ donor strain	**F^- recipient strain**	**Conjugated Sample**
LB + streptomycin	-	+	+
LB + Tetracycline	+	-	+
LB + streptomycin + tetracycline.	-	-	+

Table 2: observation of the screening

Result Interpretation:

The Donor is resistant to tetracycline and sensitive to streptomycin hence the donor will grow on LB + Tetracycline but not grow on LB + streptomycin and LB + streptomycin + tetracycline. The Recipient is resistant to streptomycin but sensitive to tetracycline hence it will grow on the LB + streptomycin but show no growth on LB + tetracycline and no growth on LB + streptomycin + tetracycline. 'Conjugated Sample' will grow on Tetracycline and Streptomycin LB plate. This is because there is transfer of antibiotic resistance gene from F factor of donor to recipient via the process of Conjugation. On the other hand, both the parental strains separately do not grow on the double antibiotic plate as it contains one other antibiotic to which they are sensitive. Bacterial lawn is also observed on plating 'Conjugated sample' will also grow on individual antibiotic plates.

Discussion:

The present study successfully demonstrated bacterial conjugation in *Escherichia coli* by transferring tetracycline resistance from an F^+ donor strain to an F^- recipient strain through direct cell-to-cell contact. The results obtained after antibiotic screening confirmed the occurrence of horizontal gene transfer, as growth was observed on the double antibiotic plate containing both tetracycline and streptomycin. This indicates that the recipient cells acquired the resistance gene carried by the donor's conjugative plasmid, a finding consistent with earlier reports by (Phornphisutthimas *et al*. 2007; Bennett 2008).

Conjugation is a major mechanism of horizontal gene transfer among bacteria, enabling the rapid spread of genetic traits such as antibiotic resistance. The process depends on a conjugative plasmid carrying the tra (transfer) genes, which encode proteins responsible for pilus formation, DNA nicking, and strand transfer (Willetts & Wilkins, 1984; Llosa *et al*. 2002). Structural studies have revealed that the Type IV secretion system (T4SS) mediates plasmid transfer by forming a transient intercellular bridge between donor and recipient cells (Cabezón *et al*., 2015; Ilangovan *et al*., 2015; Christie, P. J., *et al. 2014*). The observed transfer of the tetracycline-resistance gene in this experiment thus reflects the conserved molecular machinery underlying bacterial conjugation.

The findings also highlight how antibiotic pressure can enhance conjugation efficiency and select for resistant populations. As demonstrated in previous research, exposure to antibiotics can act as a selective driver for plasmid exchange and persistence in bacterial communities (Lopatkin *et al.*, 2016; Alalam *et al*., 2021; Beaber, J. W. *et al.* 2004). Therefore, understanding conjugation dynamics is essential for developing strategies to minimize antibiotic-resistance dissemination in clinical and environmental settings.

Beyond its biological significance, bacterial conjugation plays an important role in recombinant DNA technology. Conjugative plasmids have been widely used as tools for gene transfer, cloning, and strain engineering in biotechnology (Clark & Adelberg, 1962). The experiment reinforces the principle that plasmid-mediated DNA transfer can be exploited for controlled genetic manipulation, making conjugation not only a key evolutionary process but also a cornerstone of modern molecular biology.

Overall, the experiment provided both theoretical and practical insights into the mechanism of bacterial conjugation. The successful observation of recombinant colonies confirms the efficiency of conjugation under laboratory conditions and supports earlier models of plasmid-mediated gene exchange by bacterial conjugation (Cabezón *et al*., 2015).

Conclusion:

The above study states the successful demonstration on conjugation technique by GeNie kit, proves effectively in understanding, and explains the role of antibiotic resistance gene containing Plasmid transfer among donor and recipient bacterial strain. Also the finding supports the role of conjugation in horizontal gene transfer among bacteria and how it can demonstrate the antibiotic resistivity among bacteria, and cause the antibiotic treatment to fails in some medical cases.

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