Bacterial conjugation

Bacterial conjugation (in this mechanism bacterial DNA transfer by direct contact , cell to cell) , is the most widespread mechanism in the world of prokaryotic .

Historical events

This process was discovered by Joshua Lederberg and Edward Tatum in 1946 (Figure 1 and 2). They were studying strains of *Escherichia coli* that had different nutritional requirements for growth. A **minimal medium** is a growth medium that contains the essential nutrients for a wild-type (nonmutant) bacterial species to grow. A strain that cannot synthesize a particular nutrient and needs that nutrient to be supplemented in its growth medium is called an **auxotroph.** By comparison, a strain that could make all essential nutrients needed for growth would be termed as **prototroph**.

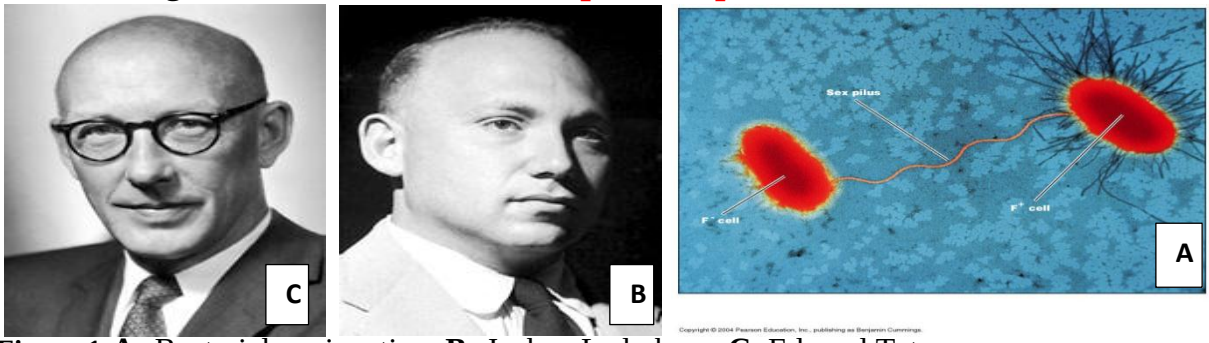

Figure 1:A; Bacterial conjugation **.B;** Joshua Lederberg .**C**: Edward Tatum.

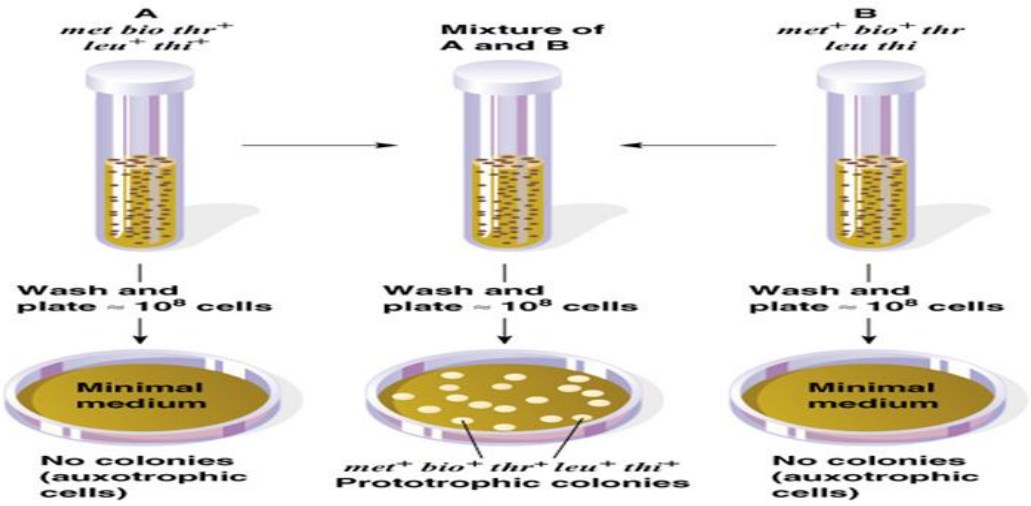

Figure 2: Tatum and Lederberg's experiment (1946) show sexual recombination that occurs between cells of *E. coli.* Parental strain (A & B) were auxotrophic: they could not grow on minimal medium : a) one strain, designated *met – bio – thr + leu + thi +*, which required one amino acid, methionine (met), and one vitamin, biotin (bio), in order to grow. This strain did not require the amino acids threonine (thr) or leucine (leu), or the vitamin thiamine (thi) for growth. b) Another strain, designated *met + bio + thr – leu – thi –*, had just the opposite requirements. It was an auxotroph for threonine, leucine, and thiamine, but a prototroph for methionine and biotin. In the middle: prototrophic strain.

In **1950**, **Bernard Davis** conducted experiments showing that **two strains** of bacteria must make **physical contact** with each other to **transfer genetic material**.

1. The apparatus he used, known as a U-tube. At the bottom of the U-tube is a filter with pores small enough to allow the passage of genetic material (i.e., DNA molecules) but too small to permit the passage of bacterial cells.
2. On one side of the filter, Davis added a bacterial strain with a certain combination of nutritional requirements (the *met – bio – thr + leu + thi* + strain).
3. On the other side, he added a different bacterial strain (the *met + bio + thr – leu – thi* – strain).
4. The application of alternating pressure and suction promoted the movement of liquid through the filter.
5. Because the bacteria were too large to pass through the pores, the movement of liquid did not allow the two types of bacterial strains to mix with each other.
6. However, any genetic material that was released from a bacterium could pass through the filter.
7. After incubation in a U-tube, bacteria from either side of the tube were placed on media that could select for the growth of cells that were *met + bio + thr + leu + thi* +. These selective media lacked methionine, biotin, threonine, leucine, and thiamine, but contained all other nutrients essential for growth.
8. In this case, no bacterial colonies grew on the plates.(Figure 3).

Conclusion: The experiment showed that, without physical contact, the two bacterial strains did not transfer genetic material to one another.

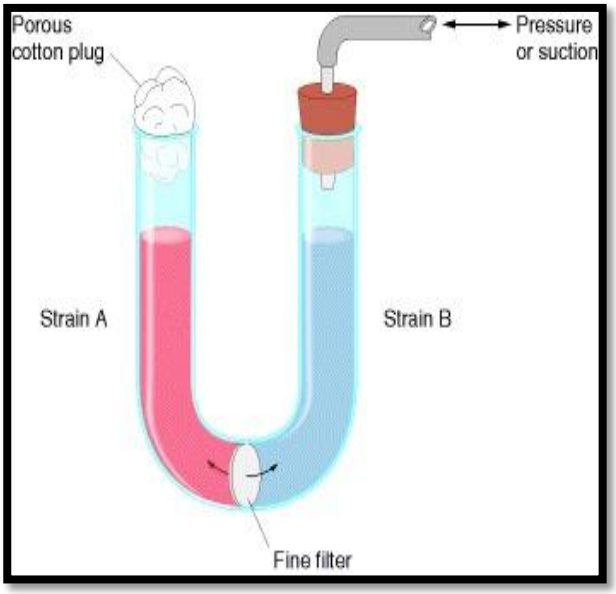

Figure 3: A U-tube apparatus like that used by Bernard Davis.

Procedure

Materials:

1 lyophilized vial contain strain A: *E. coli* as Donor cell (F+)/Tetracyclic resistant
1 lyophilized vial contain strain B: *E.coli* as Recipient cell (F-)/ Streptomycin resistant
Streptomycin
Tetracycline
LB Agar Plates (2 plate for each group)
Streptomycin / Tetracycline LB Agar Plate (one plate for each group)
Forceps
Centrifuge Tubes
Inoculating Loop

Precautions:

1. All the ingredients used in the process of conjugation should be made perfectly sterile.
2. Both the strains of bacteria should reach the desired OD at 600 nm before executing the conjugation experiment.

Day1: Revival of the parental strain

1. Break open one set of lyophilized vials (donor and recipient *E.coli* strains). Rehydrate each vial with 0.1 ml of sterile LB broth.
2. Streak the donor strain on LB plate with tetracycline (concentration 30 µgm/ml) and the recipient strain on LB with Streptomycin (concentration 100 µgm/ml).
3. Incubate the plates at 37˚C overnight.

Day 2:

4. Pick the single colony each from the donor and recipient plate and inoculate into 6 ml LB broth containing the respective antibiotic
5. Incubate at 37˚C in a shaker overnight.

Day 3: Conjugation

6. Inoculate 1 ml of overnight donor culture into 25 ml LB broth (in 250 ml conical flask) with tetracycline at a concentration of 30 µgm/ml.
7. Incubate at 37˚C in a shaker.
8. Inoculate 3 ml of overnight recipient culture into 25 ml LB broth (in 250 ml conical flask) with streptomycin at a concentration of 100 µgm/ml
9. Incubate at 37˚C in a shaker.
10. Grow recipient and donor cultures till the O.D. of the donor culture reaches 0.8-0.9 at A 600.
11. Take 0.2 ml each of donor and recipient cultures in a sterile cotton plugged test tube for conjugation. Label this as conjugated sample.
12. Gently mix and incubate in an incubator at 37˚C for 1-2 hrs.

Note: Do not place the tubes in a shaker during conjugation period.

13. Spread 0.1 ml of each of the samples (donor, recipient, conjugated sample) on antibiotic plates as indicated in the table below.
14. Incubate the plates at 37˚C overnight.

Day 4:Observation

	LB+Streptomycin	LB+Tetracycline	LB+Strept+ Tetracycline
Donor Strain A			
Recipient Strain B			
Conjugated sample			

Discuss the results:

From the observations we can interpret that:

1. Donor and the recipient grow only on those antibiotic plates to which they are resistant to.

2. Donor and recipient being sensitive to Streptomycin and Tetracycline respectively will not grow on those antibiotic plates.

3. Conjugated sample grow on tetracycline and streptomycin LB plate. This is because there is gene transfer of antibiotic resistance from F factor of donor to recipient via the process of conjugation. On the other hand, both parental strains do not grow on the double antibiotic plate as it contains one or the other antibiotic to which they are sensitive.