Phermone-inducible conjugation in *Plesiomonas shigelloides*

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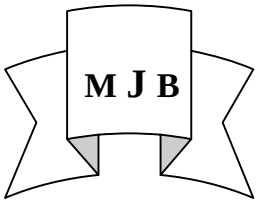

Summary:

A method of bacterial conjugation was developed using sex pheromone extracted from *Enterococcus faecalis*. Bacterial conjugation was proceed between isolate of *plesiomonas shigelloids* (isolated from diarrhea case) which was resistant to tetracycline and *Escherisia coli* MM 294 Which was resistant to rifampicin and has no plasmid. Bacterial conjugation in these bacteria failed in ordinary method while it succed in the developed method and the results showed that the conjugant cells of *E. coli* MM 294 acquired tetracycline resistance feature which is the characterized feature of the donor strain.

الخلاصة:

تم استحداث طريقة للاقتران البكتيري وذلك بأضافة الفيرمون الجنسي المستخلص من سلالة *Enterococcus faecalis* لاجراء الاقتران البكتيري بين عزلة واحدة من بكتريا *Plesiomonas shigelloides* المعزولة من حالة اسهال كسلالة واهية للبلازميد والمقاومة للتتراسايكلين الى سلالة *Escherisia coli* MM 294 الخالية من البلازميد والمقاومة للريفامبيسين علماً ان عملية الاقتران في هذه البكتريا لم تنجح بالطريقة الاعتيادية واظهرت النتائج ان الخلايا المقترنة من بكتريا*E.coli* MM 294 قد اكتسبت صفة المقاومة للمضاد الحيوي التتراسايكلين المميز للسلالة الواهية.

Introduction:

Sex phermones are small (7-8 amino acid) peptides of low molecular weight (less than 1000 daltons). Sex phermones are extracellular which are encoded by the chromosome [1,2].

(Dunny et al.)[3] extracted 20 phermone-response plasmids and they indicated that sex phermones play important role in transferring of the phermone-response plasmids responsible for antibiotic resistance and virulence factors by bacterial conjugation.

Phermones is extracellular chemical signal sends form the bacterial cells to induce the bacterial conjugation between the donor and recipient cells and increase the transferring of genes among different bacterial genera and this called horizontal exchange of the plasmids [4,5] .

Transferring of plasmids by pheromone occurs in steps:

1. Phermone induces pheromone responsive gene which leads to creation of pheromone receptor on the surface of donor cells in which pheromone binds.
2. Binding of the pheromone in this receptor induce entrance of pheromone to inside of the donor cells by oligopeptie- permase which provide pores in the cellular wall that permits pheromone entrance.
3. Phermone induces donor cells to synthesize two types of proteins on the donor cells surface which are aggregation substances and Exclusion substances.
4. Aggregation substances on the donor cells bind with Binding substances (encoded by chromosome) on the recipient cells, this binging make donor and recipient cells in direct

contact then followed by formation of matting canal between throw donor and recipient cells.

5. The entrance pheromone induces genes on the responsive plasmid for replication and creation of new copy of plasmid which will transferred from the donor cells to recipient cells throw the matting canal.
6. After transferring and stability of the plasmid in the recipient cells there are two ways act on finishing of conjugation:

A- Reduction of sex pheromone production secreted by recipient cells.

B- Donor cells synthesize pheromone – inhibitor which competes with pheromone on binding in pheromone receptors present on the donor cells surfaces[6,7,8].

Aim of the study:

The possibility of bacteria to success in bacterial conjucation using sex pheromone.

Materials and Methods

A- Extraction of sex pheromone

Extraction of sex pheromone was achieved according to method of (Buttaro et al) [2] as in the following steps:

1- The standard strain *Enterococcus faecalis* FA2-2 that produced sex pheromone cCF10 was cultured in 500 ml of Todd-Hewit broth and incubated at 370°C for 18 hours.
2- The bacterial culture was centrifuged at 8000 round/minute for 10 minutes.
3- The supernatant was autoclaved for 15 minutes, then 5% of trichloroacetic acid was added to supernatant in order to precipitate the pheromone.
4- The precipitate was suspended with 0.2 ml of phosphate buffer saline, pH was adjusted to 7, then the volume was completed to 0.5 ml. these steps were repeated several times in order to obtain sutible amount of pheromone.

B-Phermone-inducible conjugation:

1- Isolate of *Plesiomonas shigelloides* (isolated from diarrhea case in maternity and children hospital in Hilla province) resistant to tetracycline (10 mg/ml) and sensitive to rifampicin (100 mg/ml) was used as donor strain, the standard strain *E. coli* MM 294 (Biotechnology department /college of science/university of Baghdad) that has no plasmid and has resistance feature to rifampicin on the chromosome was used as recipient strain.

2- The donor and recipient strains were cultured in nutrient broth and incubated at 37°C for 4-5 hours.

3- Equal volumes of sex pheromone and nutrient broth were mixed in sterile tube.

4- One ml of the donor cells culture was mixed with 9 ml of mixture of (sex pheromone and nutrient broth), also 1 l of the recipient cells culture was mixed with 9 ml of nutrient broth. All tubes were incubated at 37°C for 1 hour.
5- Half ml of induced donor cells was mixed with 4.5 ml of non induced recipient cells, also 0.5 ml of non induced donor cells was mixed with 4.5 ml of non induced recipient cells. All tubes were incubated at 37°C for two hours.

6- The mixture was shaked well by vortex for one minute to separate the donor cells from the recipient cells.

7- Conjugation mixture was diluted ten fold dilutions, then 0.1 ml of each dilution was spread on the brain – heart infusion agar contained (10 mg/ml) of tetracycline and (100 mg/ml) of rifampicn to determine the total number of conjugant cells. 0.1 ml of each dilution was spread on the brain-heart infusion agar contained (10 mg/ml) of tetracycline only to determine the total number of donor cells and 0.1 ml of each dilution was spread on the brain-heart infusion agar contained (100 mg/ml) of rifampicin only to determine the total number of the recipient cells. 0.1 ml of origin culture of donor cells was spread on the brain-heart infusion agar contained (100 mg/ml) of rifampicin only to determine the mutant cells spontaneously and 0.1 ml of the origin culture of recipient cells was spread on the brain-heart infusion agar contained (10 mg/ml) of tetracycline only. All plates were incubated at 37^{o}C for 24 hours.

8- Number of colonies were picked again and cultured on brain-heart infusion agar without antibiotics, then they were cultured on brain-heart infusion agar contained antibiotics (10 mg/ml) of tetracycline and (100 mg/ml) of rifampicin to confirm the fixation of the antibiotic resistance feature and continuous of its inheritance.

9- Plasmid content of the conjugant cells was studied to detect the transferred plasmid from the donor cells to recipient cells.

10- Plasmid DNA was extracted by salting out method [9].

11- Method of (Sambrook et al.)[10] was followed in the electrophoresis of plasmid DNA to detect DNA bands by UV translluminator at wave length 302 nm.

Results and Discussion:

Phermone-inducible conjugation was procced for *P. shigelloides* isolate which used as donor strain of plasmid to recipient strain *E. coli* MM 294 which has no plasmid and resistant to rifampicin.

After culturing in the media contained rifampicin and tetracycline, results showed that conjugant cells acquired tetracycline resistance feature which indicate that the genes responsible for tetracycline resistance feature present on the plasmid that transferred by pheromone-inducible conjugation to recipient strain strain *E. coli* MM 294 (figure 1).

The plasmid did not transfer by ordinary bacterial conjugation and this indicates that *P. shigelloides* has conjugant plasmid which transferred to recipient strain by adding th sex pheromone.

Phermone-inducible conjugation occurs in nature in frequency particularly among Gram positive bcteria that resistance to tetracycline such as *Enteroccus* and *Streptococcus* [11].

Sex pheromone can increase frequency of bacterial conjugation among different bacterial genera, where sex pheromone increase the direct contact between the dono and recipient cells and transferring of genes responsible for resistance of antibiotics [12]. pheromone is

considered clumping inducing agent also it increase fertility of the bacteria which increases the conjugation that lead to transferring of pasmids responsible for antibiotic resistance, hemolysin production and bacteriocin production.

Phermone encocurges formation of matting channel throw which transferring of plasmids of virulence occurs.

Phermone inducible conjugation occurs in nature in high frequency among the species even in species that conjugation did not occurs in them ordinarily [13; 14].

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Figure (1) Phermone-inducible conjugation in *Plesiomonas shigelloides*
1-Recipient strain.
2- Donor strain *Plesiomonas shigelloides.*
3-Standard strain *E. coli* MM 294.