COMBINED MATING TECHNIQUE FOR ASSESSING CONJUGAL TRANSFER BETWEEN TEST VIBRIO CHOLERAE 01 MZW 41 AND PSEUDOMONAS AERUGINOSA W 109[+]

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Abstract

This study presents results of comparing four in vitro conjugal transfer techniques, colony cross streak (CCS), broth mating (BM), combined spread plate (CSP), and membrane filtration (MF) with each other in evaluating the transfer of cojugative plasmids. Vibrio cholerae 01 MZW 41 and Pseudomonas aeruginosa W109, were used as mating pairs.

The suitability of each method was evaluated for use in a routine assessment of the genetic stability of genetically transconjugated microorganism. By the BM technique, transconjugants were usually produced in 100% of the mating traits. In general, differences in the percentage of successful mating show that BM technique is statically significant at the $P \leq 0.05$ level, compared with CCS, CSP, and MF techniques. Since the genetic exchange between Vibrio and Pseudomonas was not detected previously (an exception to this, what has been reported by Al – Amili, W.A. (2001), and any single conjugation transconjugants. We have used a combined mating technique which integrates the CCS, CSP, BM, and MF method as a single procedure to assess the mobility of plasmid DNA of genetically transconjugated microorganisms.

المستخلص

تظهر هذه الدراسة نتائج مقارنة أربعة تقنيات إنتقال إقتراني في الزجاج هي، التخطـيط المتقـاطع للمستعمرات (CCS)، التزاوج بالسائل (BM)، طبق النشر المركب (CSP)، والترشيح الغـشائي (MF) مع بعضها في تقييم إنتقال البلازميدات الاقترانية. بكتريا ضمات الكوليرا 01 MZW 41 و بكتريا الزائفـة الزنجارية W109 أُستخدمت كأزواج تزاوج. ملائمة كل طريقة قيمت للاستخدام في التقدير الروتيني للثبات الوراثي للميكروبات المقترنة وراثياً.

بإستخدام تقنية BM، انتجت المقترنات عادة نسبة 100% لخواص التـزاوج ، بـصورة عامـة. الاختلافات في النسبة المئوية للتزاوجات الناجحة اظهرت ان تقنية BM كانت معنوية احصائياً عند مستوى $P \leq 0.05$ مقارنة مع تقنيات CCS, CSP و MF . بما ان التبادل الوراثي بين بكتريا الضمات وبكتريـا الزائفة لم يلاحظ مسبقاً (عدا ما نشر من قبل (AL – Amili, W.A. (2001). و إن اي تقنيـة تـزاوج اقتراني مفردة لا تكون موثوقة بصورة تامة في ملاحظة المقترنات، إستخدمنا تقنية مركبة للتزاوج والتـي

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تكامل الطرق BM, CSP, CCS, و MF كطريقة عمل مفردة لتقدير قابليـــة تحـــرك الـــدنا البلازميـــدي للميكروبات المقترنة وراثياً.

Introduction:

Plasmids are important in medicine because they confer antibiotic resistance on pathogens of animal and man, and because they can code toxins and other proteins which increase the virulence of these pathogens. To make matter worse, bacterial pathogens have become increasingly resistant to a variety of antibiotics. A major driving force behind the increase in resistant strain is the case with which bacteria can acquire resistance gene even from distantly related genera [1,2].

Cholera is a serious diarrheal disease used by intestinal colonization with toxigenic vibrio cholerae. The organisms are easily spread by contaminated water and food, and epidemics of cholera frequently appear in developing parts of the world [3, 4, 5].

On the other hand Pseudomonas species have attracted increasing attention from bacterial geneticists. The main reasons are their widespread occurrence, their biological and medical importance, their nutritional and biochemical versatility, and the simplicity of the conditions required for their cultivation in the laboratory. Of all Pseudomonas species, by far the best known from the genetical point of view, is Pseudomonas aeruginosa [6].

Multiresistance in V. cholerae has so far been linked to conjugative plasmids, and such plasmids have been reported to the transferable to Escherichia coli [7, 8]. However, few data are available concerning transfer of antimicrobial resistance from V. cholerae to P. aeruginosa.

Our rationale to adopt this strategy is to compare four established laboratory techniques for producing transconjugants.

Materials and Methods

Bacterials Strains

The bacterial strains used in this work are listed in Table 1.

Media and Cultures

Working cultures were maintained on Luria – Bertani (LB) agar and broth [9] containing antibiotic to which they were resistant. Media selection of trasconjugants for crosses containing: (GM: 32µg / ml), (S:64 µg/ml), (SXT: 16 – 256µg / ml), (PG: 16µg / ml), (AMP: 64µg / ml), (E: 32 µg / ml). plates were incubated at 30 ± 2C° and examined after 24 and 48h.

Table (1): Bacterial Strains Utilized in this Study.

Species	Source	Genotype [a]
V. cholerae 01MZW 41	Al – Amili, W.A. [b] University of Baghdad	GM S SXT
P. aeruginosa W 109	Al – Amili, W.A. University of Baghdad	PG AMP E

a GM, S, SXT, PG, AMP, and E designate resistance to gentamycin, streptomycin, co – trimaxazole (Trimethoprim – sulfamethaxazole), penicillin – G, Ampicillin, and Erythromycin, respectively.

b Al – Amili, W. A. (2001), reported that self – transmissible large plasmid (PWL) has been transferred from V. cholerae 01 MZW 41 to P. aeruginosa W 109 at a frequency rate 3.51×10^{-6} carrying a resistance markers for S and SXT using conjugal transfer by BM technique.

Mating Procedures

In the colony cross streak technique (CCS), separate cultures of donor and recipient were incubated at 30 ± 2 C° on agar containing antibiotics to which they were resistant. A selective agar plate was then streaked, first with the recipient and then with the donor, as illustrated in the upper right – hand corner of Figure 1. The donor only was streaked on one sector and the recipient only was streaked on the other, to serve as negative controls.

Broth mating (BM) [10]: Donor and recipient were incubated for 24h at 30 ± 2C° with shaking in 5ml of LB broth containing antibiotics to which both were resistant. Both were washed by centrifugation with sterile 10mM tris buffer (PH 7.5) and suspended in 5ml of the same buffer.

One ml liquot of both donor and recipient was inoculated into 5 ml of LB broth incubated statically at 30 ± 2C° for 24h. After incubation period, the mixture was shaken vigorously for 2min in a vortex mixer to separate the cell from each other.

Then, serially diluted in Tris buffer up to 10^{-7} and then 0.1 ml aliquots were spread into selective media containing the appropriate antibiotics for transconjugants selection. Control of both donor and recipient cells was treated exactly in the same manner as above to check spontaneous mutation frequencies (Figure 1).

The combined spread plate (CSP) technique was a modified version described by Tardif and Grant(1983). Donors and recipients were incubated under conditions described for BM. A 1.5ml aliquot of each culture was microfuged and washed in Tris – buffer, and suspended in 1.5 ml of the same buffer. A 100 µl aliquot of the recipient was spread plated onto a selective agar plate and allowed to dry for 5min, and a 100 µl aliquot of the donor was the spread plated over the recipient.

The membrane filtration (MF) technique was modified from the procedure described by Chatterjee and Starr(1973). Donor and recipient cultures were incubated and washed as described for BM. Aliquot 1ml, of donor and recipient suspensions were added to 10ml of Tris buffer (PH 7.5) in a screw – cap test tube and then filtered through a 0.45 µm nitrocellulose filter (millipore crop.). Filters were transferred onto LB agar plates and incubated for 3h at 30 ± 2C, removed from plates, and vortexed for 1min in 1ml of Tris buffer (PH 7.5). Serial dilutions were then spread plated onto selective agar plates and incubated.

Mating rates were expressed as transconjugants produced per initial recipient cell at the beginning of the incubation period.

Successful mating among the four mating techniques were evaluated by chi – square statistical analyses.

Extraction of Plasmid DNA

The large – scale plasmid extraction and purification [13] was used for extraction of plasmid DND from the presumptive transconjugants. Plasmids were visualized by using standard technique [14] following horizontal electrophoresis in 0.7% agarose gels and staininpg with 0.5µg of ethedium bromide per ml.

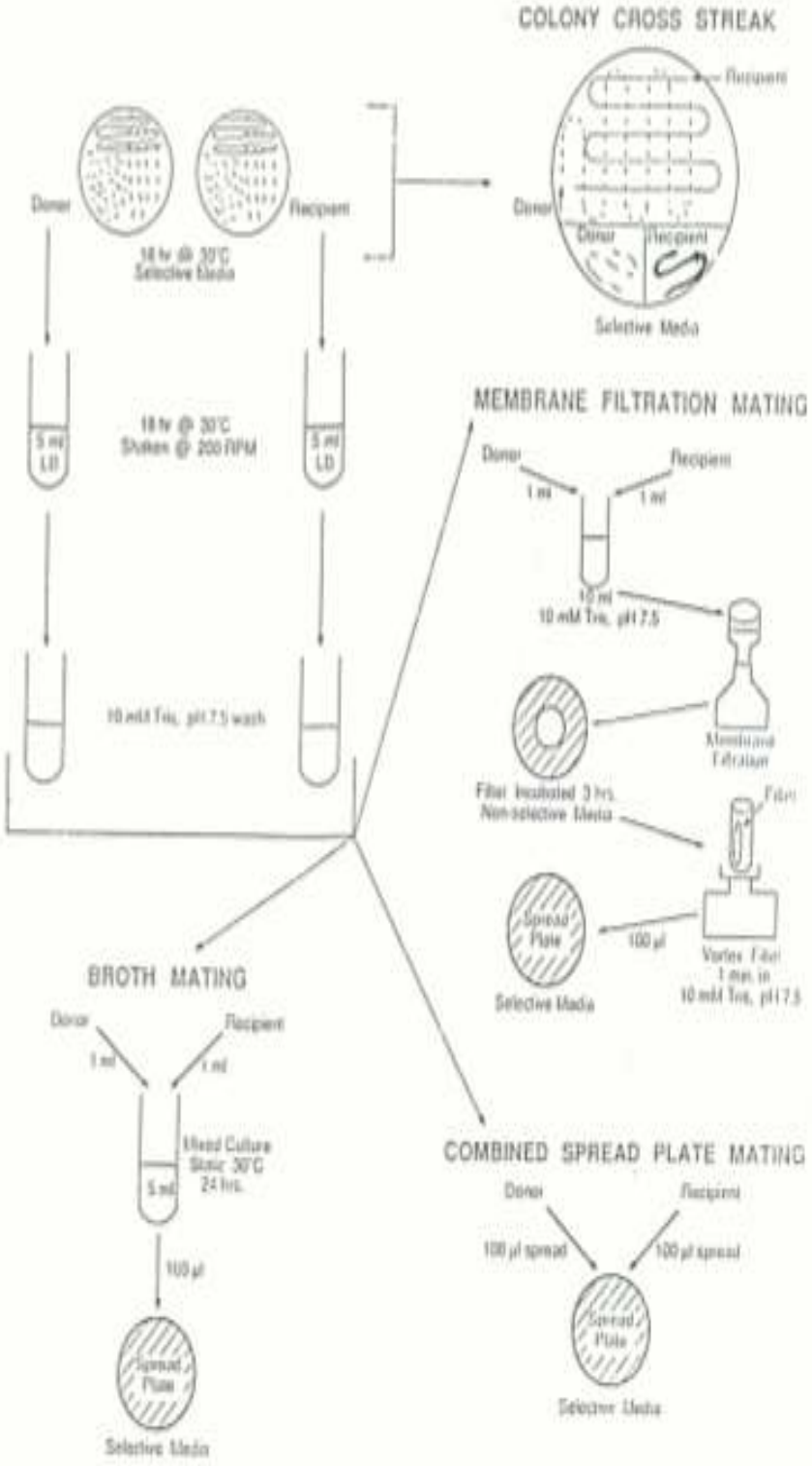

Figure (1): Brief Combined Mating Technique Scheme *

* A single source of washed cell suspensions is utilized executing the BM, CSP, and MF technique

Results

The results of the four techniques used to demonstrate conjugation are compared in Table 2. Each technique was capable of demonstrating transconjugant formation.

The BM method produced transconjugants most consistently. The BM demonstrated a higher success rate than the three other methods. The comparisons of BM with CCS, CSP, and BM regarding to the percentage of successful matings are statically significant at the $P \leq 0.05$ level.

Table (2): Percentage of successful mating by the four conjugal mating techniques.

Donor	Recipient	% successful mating by *			
		CCS	BM	CSP	MF
V. cholerae 01 MZW 41	P. aeruginosa W 109	38	100	60	45

* Each value represents the average of at least 10 experimental replications

The frequencies of mating of the mean expressed as transconjugants per recipients observed for each mating technique are listed in Table (3).

Table (3): Comparison of transconjugation mating frequencies by mating technique.

Donor	Recipient	Mating frequency (transconjugnats / recipient)*			
		CCS	BM	CSP	MF
V. cholerae 01 MZW 41	P. aeruginosa W109	4.9×10^{-9}	3.51×10^{-6}	2.7×10^{-7}	1.4×10^{-8}

* Each value represents the average of at least 10 experimental replications.

The highest frequencies of transconjugation were observed with the BM method.

Discussion

Acquired resistantce involves the DNA content of a cell, such that the cell acquires a phenotype (i.e. antibiotic resistance) which is not inherent in that particular species the spread of antibiotic resistance by transmission of genes coding for resistance to individual drugs from resistant strains to susceptible ones seems to contribute in general to the increase of bacterial resistance to individual antibiotics [2].

As a result various methods have been developed to detect and evaluate in vitro conjugation events. The conjugal mating techniques utilized in this study were selected because they are representative of the diverse methods found in the literature. This study attempts to compare these methods in term of their sensitivity and reliability in side – by – side tests and to evaluate their suitability as preliminary screening techniques in testing epidemiological strains (as V. cholerae) routine screening of donor and recipient microorganisms requires a method which is sensitive and provides reproducible results, but is also cost and time effective.

The CCS technique is quick, simple, and inexpensive to perform. As a result, a large number of samples can be tested and replicated. However, because this technique utilizes donor and recipient cultures cross streaked onto a solid medium, it is not quantitative.

The BM technique is as reliable as the CCS but has the advantage of being quantitative. The results obtained with this method were in general agreement with results reported by others [15, 16].

The CSP technique is also quantitative and simple to perform, and many donor and recipient cultures can be screened rapidly. Tardif and Grant(1983) demonstrated transfer rates of 9×10^{-7} by the CSP technique with the plasmid R 40a and P. aeruginosa recipient.

MF is the most reliable of the four methods compared. While it is very reliable, the MF technique is more time – consuming and requires additional glassware and laboratory equipment. It would not tend it self well to use in routine screening of large numbers of donor and recipient or in the replication of measurements.

Unfortunately, relatively few studies of this type have been done, and because no single mating technique is capable of demonstrating superior results with all species were used as donor and recipient, therefore an integral procedure was used to incorporate be CCS, BM, CSP, and MF techniques in a single process for evaluating conjugal transfer of DNA (Figure 1) this procedure is designated to combin mating technique. One source of washed cell suspensions can be used to execute the quantitative procedures (BM, MF, and CSP).This combined matting technique provides both liquid and solid substrates for mating. This increases the chances of detecting transconjugants since certain plasmids transfer preferentially when mating pairs are incubated on solid surfaces [12].

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