Modifying Plaque assay and Clearance test as tools in determination of phage typing for *E. Coli* bacterial interspecies

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Abstract :

Bacteriophage of *E. Coli* interspecies from sewage samples were isolated , the phage particles were isolated from two different sewage samples . The first sample was collected from sewage sample of Baghdad university and the second sample was isolated from domestic sewage sample , first sample showed phages specialized for three *E. Coli* interspecies bacteria (first plate) and two *E. Coli* interspecies bacteria (second plate) , meanwhile second sample showed phage specialized for two *E. Coli*. interspeciesThe study of appearance of *E coli* phages from first sample showed three types of *E. coli* phages with different size of inhibition zone (1 , 0.7,0.5)Cm respectively (first plate) , meanwhile *E. Coli* interspecies bacteria showed phages related with two interspecies with size of inhibition zone (0.5 ,0.4) Cm respectively (second plate), on other hand , the second sample showed also two interspecies *E. coli* with inhibition zone (1,0.8)Cm . experimental method has been designed which showed the modifying method of phage assay to determine phage typing assay . phage has been tested particles with different bacterial strains (*E. coli , shagilla and Serratia*) from different sources and the control was the host of each bacteriophages by taking the O.D for all the tests and controls , to setup new criteria for phage typing .:and this test is called (Clearance Test) The result showed that O.D for Test 1 , 2 , 3, was (1.6 , 1.2 . 1.7) for (*E. coli , shagilla and Serratia*) bacterial strains , meanwhile the control tests was (0.3 , 0.2, 0.4) for strains isolated from first sample (first and second plate) and second samples with different interspecies respectively . This result can predict high specificity of phage strain and this method can be used to determine interspecies strains .So from this experiment we can identify only Clearance Test by measuring only O.D. of bacterial strain with different phages instead of going through plaque assay .

Key words : Phage typing , *E. Coli* interspecies , Plaque assay , Clearance test

Introduction :

Phage typing is a rapid, economical, reliable, and reproducible technique, requiring no specialized equipment, for fingerprinting disease-causing agents for epidemiological investigation and surveillance. Intraspecies differentiation of bacteria can be based on taxonomic features, such as morphology, biochemical properties(biotyping), virulence (pathotyping), and antigenic structure(serotyping). In addition, a wide variety of genome-based taxonomic techniques [1] have been developed such as pulsed-field gel electrophoresis (PFGE) [2] amplified-fragment length polymorphism (AFLP) [3] and amplification of repetitive bacterial DNA elements (REP-PCR) [4] Other typing systems are basedon sensitivity to specific chemicals, including antibiotics, plasmid profiling,

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ribotyping, and the production of or sensitivity to bacteriocins and bacteriophages (lysotyping or phage typing[5]

Phage typing provides long-term and internationally comparable surveillance data. Because of their recent introduction, such information is not available for molecular techniques. Phage typing of enteric pathogens has been and still is being used successfully to characterize disease-causing agents for epidemiological investigation and surveillance [6,7]

A functional phage typing system includes the following characteristics:

a) A panel of genetically and phenotypically stable temperate or lytic phages possessing broader rather than narrow host-range specificities.

b) Results, which are obtained quickly and are clear-cut and require limited training in interpretation.

c) Method that can be standardized.

d) Bacterial cells must display a stable phage type over time.

e) Bacterial cells form a phage lytic reactions can be easily determined[8]

The typing phages may be isolated either from the environment(sewage, river, lake water,

strains. Phage isolation from the bacterial culture is possible directly from a rapidly growing broth culture, after UV irradiation or mitomycin-c treatment

Biological features of the phages are a very important issue Lytic as well as temperate bacteriophages may be used for typing [9,10]

Goals of experiment : .

. 1 . Realize very efficient method to determine phage therapy from modifying plaque assay .

. 2 . Set up criteria to show positive and negative results of phage typing method by using Clearance Test .

3 . The typing method goes through interspecies with high accuracy result

Materials and Methods:

Modifying plaque assay

1 . Pour about 40 ml of sterile molten phage agar into each plate then Sub-culture each strain of the species to be phage typed onto an LB agar plate to obtain isolated colonies.

2. Incubate the LB plate at 37 ^{0}C overnight.

3. Pick, with a sterile inoculating needle, a small amount from the center of a smooth colony and suspend each pick in one tube of LB broth.

4. Incubate these culture tubes in a water bath shaker at 37 ^{0}C and 100 rpm for approximately 1 h our until the culture becomes slightly turbid.

5. Flood a dried phage agar plate with the phage broth culture using a disposable transfer pipette to produce a bacterial “lawn” of the test strain.

6. Dispense one drop of each phage on to the phage agar plate

7 Remove phage agar plate and allow phage drops to dry on the phage agar plate for a maximum of 15 min with the lid ajar.

8. Repeat the process with a plate that has not been inoculated with bacteria. This is the sterility check on the phage preparations. Invert and incubate the dried plates at 37 ^{0}C overnight. [8,10]

Clearance Test

1 . Take 3 ml of phage buffer in two tubes for each strain to be tested , first tube as test and second tube as control .

2 . Suspend 0.1 of each bacterial strains in control and test tubes .

3 . Add to test tubes phage particles of host bacterial strains meanwhile the addition normal saline in control tubes .

4 . All tubes are incubated for 24 hours at 37 C to show lytic activity and reduction of growth in test tubes for each bacteriophage with it's bacterial strains hosts till get clearance [10,11]

Results:

The modifying plaque assay technique showed incredible result of interspecies of *E. coli* bacterial strain from different samples

The appearance of *E coli* interspecies showed that there were three different isolated phages strains with zone inhibition (1 , 0.7 , 0.5) Cm (first plate) meanwhile there were only two different isolated phage strain with zone of inhibition (0.5 , 0.4) mm respectively (second plate) as shown in figure .(1,2)

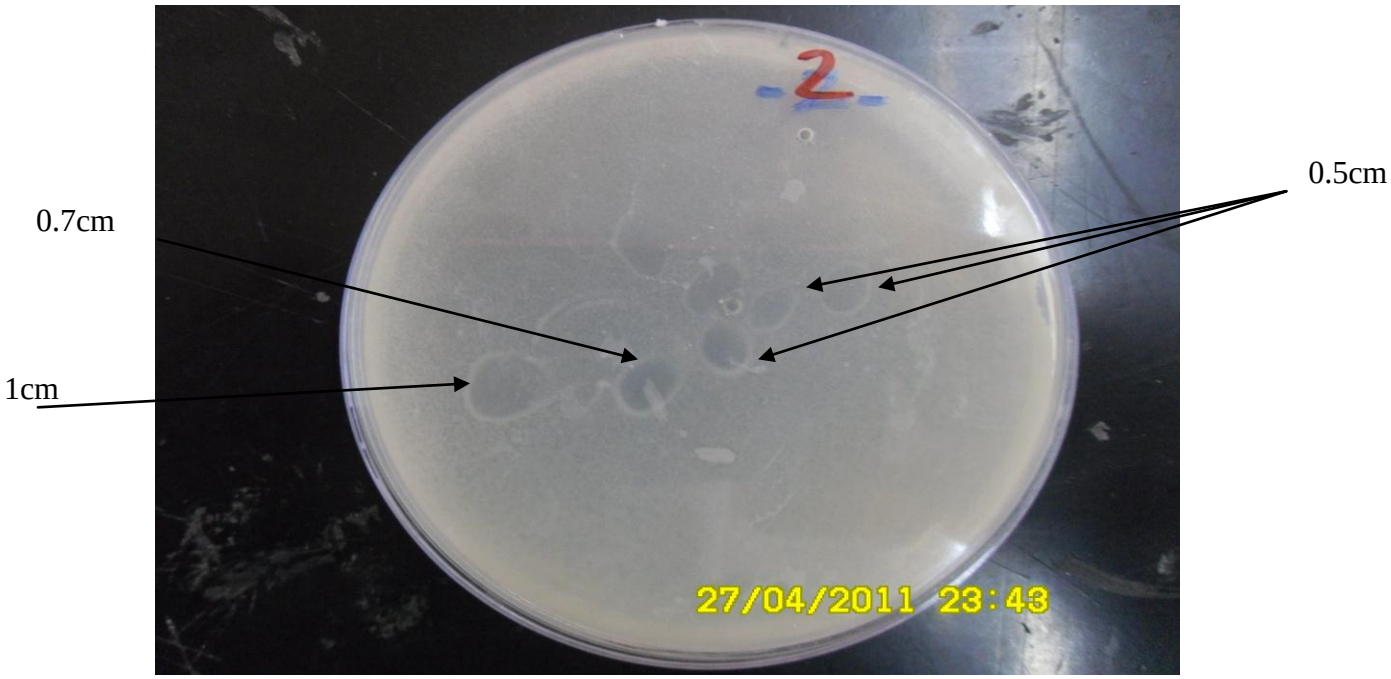

Fig. (1) : show plaque assay of bacteriophage of *E coli* interspecies for sample one (first plate)

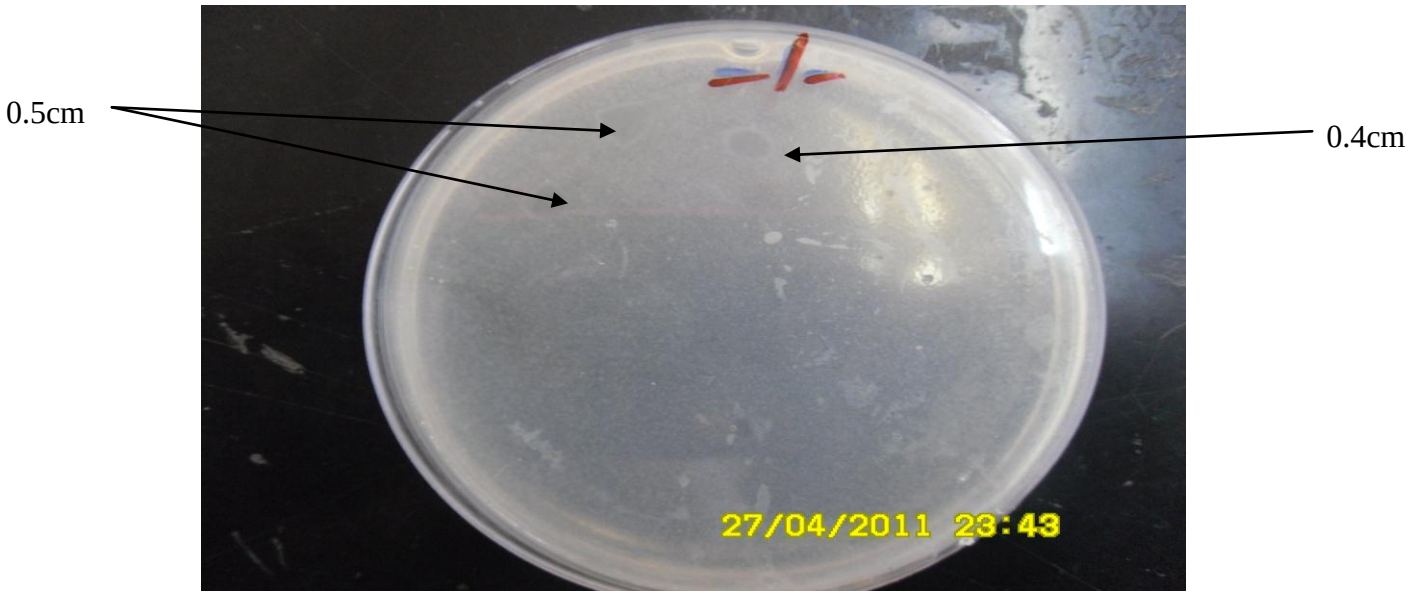

Fig. (2) : show plaque assay of bacteriophage of *E coli* interspecies for sample one (second plate)

On other hand the *E coli* phage particle of second sample show also two interspecies with appearance (1,0.8) Cm as in figure (3) .

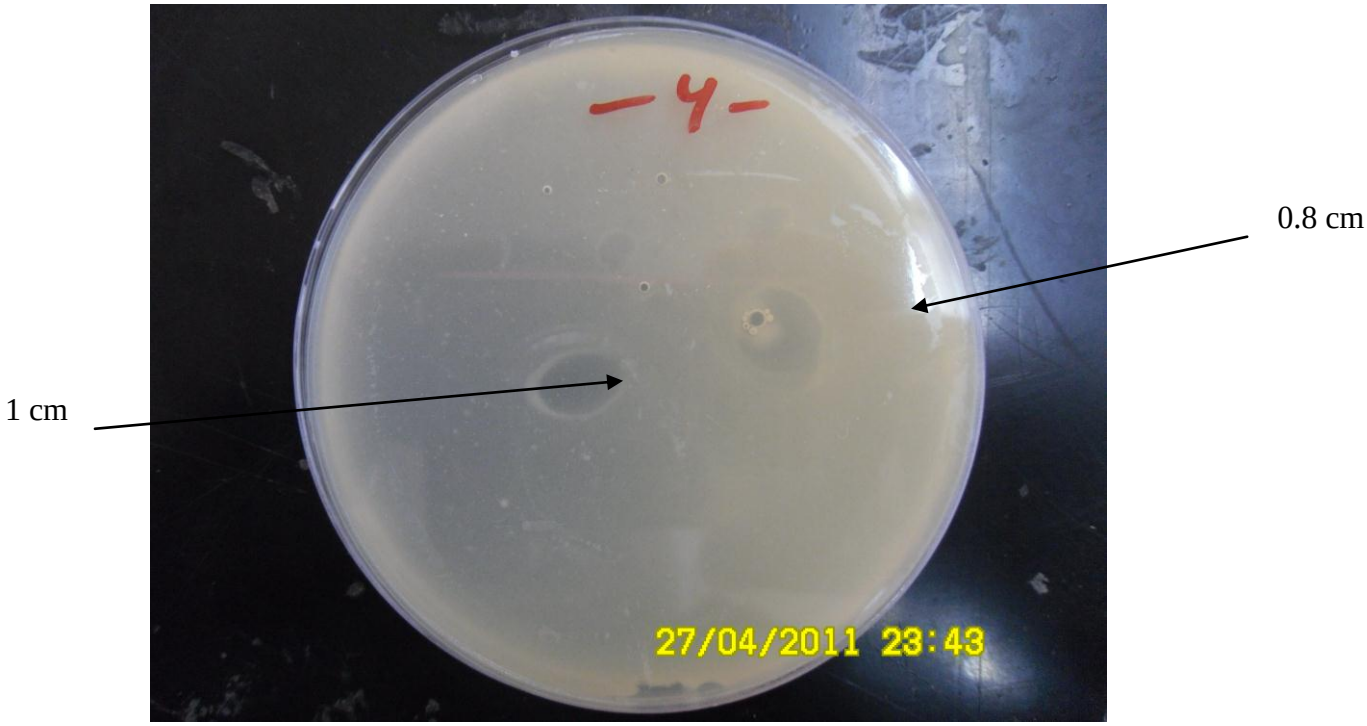

Fig. (3) : show plaque assay of bacteriophage of *E coli* interspecies for sample two

The modifying experimental plaque assay set up as test and control .
Test 1.and 2 and 3 with different bacterial strains *E coli* shagilla and Serrtia meanwhile the control 1,2,3 E coli from sample 1 (first and second plates) and sample 2 .
We set up criteria to establish new method to evaluate the modifying plaque assay by clearance test through determine the O.D. of bacterial control and tests .
The results showed that positive sample with plaque assay showed O.D. not more than 0.4 in which control 1,2,3 showed 0.3 and 0.32 and 0.38 respectively mean while the negative samples showed minimum O.D was 1 in which test 1,2,3,showed 1.6, 1.7 ,1.2 respectively as elucidated in figure (4)

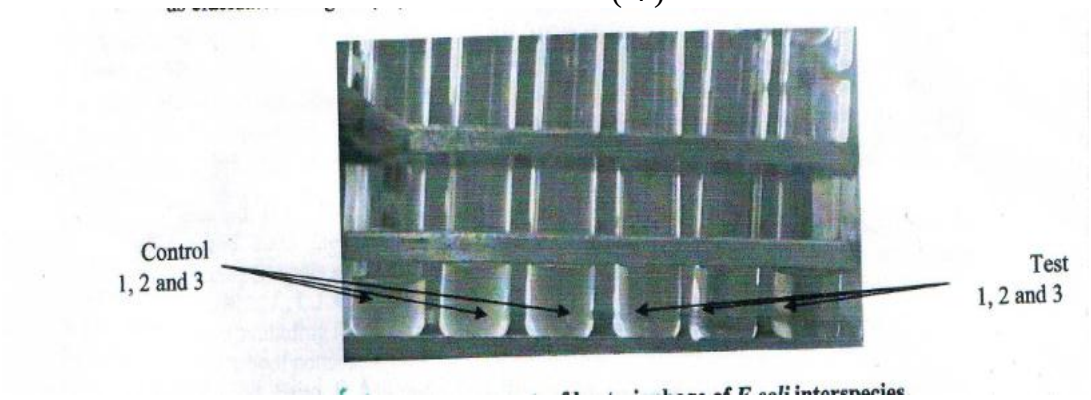

Fig. (4) :clearance test of bacteriophage *E coli* interspecies

The results dramatically significantly to determine interspecies bacteria doing clearance test instead of using more sophisticate technique like plaque assay

Discussion:

The role of phages as interspecies typing is very efficient in determine the more accuracy of related host . From our modifying plaque assay we got significantly important data which should differentiate E. coli interspecies phages particles related to first and second samples . .

The result should very significantly determination to analytical of modifying technique . Then we identify the data of modify plaque assay with normal test which named clearance test by taking O.D. of each strains of test with phage particular and control with phage of related interspecies phage particular .The results showed that positive sample with plaque assay showed O.D. not more than (0.4) in which control – 1 for (first sample – first plate) showed O. D with 0.3 , control – 2 for (first sample – second plate) showed O. D with 0.32 and control – 3 for (second sample) showed O. D with 0.38 , mean while the negative samples showed minimum O.D was 1 in which test - 1 for (E coli strain) showed O. D with (1.6) , test - 2 for (Shagilla strain) showed O. D with (1.7), test - 3 for (Serratia strain) showed O. D with (1.2) .

The reduction of O. D for positive result (control) to (0.4) as maximum meanwhile the negative result was (1) as a minimum in due to the bacteriophage in control was specific to bacterial strain in sample therefore bacteriophage lyses bacterial strain and led to reduction in number of bacterial strain then reduction in O.D. of those strain , on other meaning the lytic activity of bacteriophage to bacterial strains in control led to reduction in turbidity of growth and clearance the sample therefore we call it clearance test .

The tests showed O.D not less than (1) in due to the bacteriophage in tests was not specific to bacterial strains in sample therefore bacteriophage does not lyse bacterial strain and does not lead to reduction in number of bacterial strain .Therefore the tested tubes were turbid with high value of O.D.

We conclude that the modifying plaque assay is an incredible technique to isolate different phages form different samples and use those bacteriophages to identify not only the difference between bacterial strains but also the difference between interspecies of different strains in very specific manner by Clearance test .

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طريقة تبقع الفاج المحورة وفحص الشفافية كأدوات في تحديد نمط الفاج لبكتريا اي كولاي تحت الانواع

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الخلاصة :

فاجات بكتريا تحت النوع *اي كولاي* من عينات مياه المجاري ، العينة الاولى جمعت من عينة مياه المجاري لجامعة بغداد والعينة الثانية اخذت من مياه مجاري منزلية ، العينة الاولى كانت تحتوي على ثلاث انواع فاجات خاصة لل *اي كولاي* لتحت انواع مختلفة (الطبق الاول) ونوعين لفاجات ال *اي كولاي* لتحت النوع (الطبق الثاني) ، بينما العينة الثانية كانت تحتوي كذلك فاجين خاص لل *اي كولاي*دراسة مظهر فاجات بكتريا ال *اي كولاي* من العينة الاولى كانت تحتوي على ثلاث انواع من الفاجات مع احجام مختلفة لحلقات تثبيط (1 ، 0.5،0.7) سنتمتر للطبق الاول ، بينما فاجات ال *اي كولاي* كانت تحوي نوعين من حلقات التثبيط (0.5 ، 0.4) سنتمتر (الطبق الثاني) وكذلك اظهرت العينة الثانية نوعين من اي كولاي مع حلقة تثبيط (1، 0.8)

نحن صممنا تجربة والتي عرضت طريقة محورة لتحليل الفاج لتحدد (نوع البكتريا بأستخدام الفاج) تنميط الفاج من مصادر مختلفة بينما المحلول القياسي تمثل بمضيف المتمثل ببكتريا التي عزل منها الفاج

ومن خلال قياس الكثافة الضوئية لكل من الفحوصات والمحلول القياسي يتم تحديد نوع البكتريا وعائدتها الى فاج معين او لا وهذا الفحص سمي (فحص الشفافية) .النتائج عرضت الكثافة الضوئية للفحوص 1,2,3 كانت (1.6 ، 1.2 ، 1.7) لبكتريا ال *اي كولاي* و الشكلا والسريشيا بينما اظهرت نتائج الظابطة النسب التالية (0.3 ، 0.2 ، 0.4) للأنماط المعزولة من العينة الاولى (الطبق الاول والطبق الثاني) والعينة الثانية بأنماط مختلفة على التولي

من النتائج تبين وجود خصوصية عالية لتنميط الفاج للبكتريا المختلفة ومنه نستطيع ان نقيس نسبة الشفافية للبكتريا باستخدام فاجات مختلفة وممكن ان يعوض طريقة الاطباق