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DETECTION THE PHENOTYPIC AND GENOTYPIC ACTIVITY OF LYSOGENIC BACTERIOPHAGES ON *PSEUDOMONAS AERUGINOSA*

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Abstract

Pseudomonas aeruginosa is a widespread an opportunistic pathogen of humans, has many virulence factors and regards as a one of the most famous multidrug resistant bacteria. Phage–bacteria interaction is characterized by antagonistic coevolution and driven by resistance of bacterial hosts; that can affect multiple genes, where the rate of evolution of both partners has direct consequences for bacterial resilience and phage virulence; at the same time, it's reflected by the large genomic diversity inside phage species. This study displays the interaction between four phages and their fifty clinical bacterial hosts phenotypically by biochemical tests and molecularly by specific PCR and RAPD-PCR. The results show that only four bacterial strains among fifty were affected with these four phages.

Key words: Phage, *Pseudomonas aeruginosa*, PCR, RAPD

Introduction

Viruses that infect bacteria (bacteriophages) are able to impact bacterial dynamics, bacterial genome evolution and environment biogeochemistry system. These effects vary depending on whether phages establish lytic or lysogenic infections. Although the first produces virion progeny, with lytic infections resulting in cell death, phages undergoing lysogenic infections replicate with cells without producing virions (Howard-Varona et al 2017); instead, the viral genetic material enters an inactive as "prophage" state. During that, it is inserted into the bacterial chromosome. So, lysogenic is characterized by integration of the phage genetic material into the host cell genome. Incorporated phage genome will be replicated along with the host bacteria genome and new bacteria will inherit the viral genetic material. Such transition of viral genome could take place through several generations of bacterium without major metabolic consequences for it (Kelly and Heidi, 2018). When this bacteriophage infects new bacteria, it transfers that piece of DNA in to recipient bacterial cell by mechanism called transduction (Clokie and Kropinski, 2009).

P. aeruginosa is an opportunistic pathogen of humans; generally, is widespread in the environment and commonly present in moist locations of hospitals. It causes diseases in humans with abnormal host defenses (Török *et al.,* 2017); it's a main causer of nosocomial lung infections and a common causer of wound infections, especially of thermal burns (Ryan and Ray, 2014; Riedel *et al.,* 2019).

Commonly, lysogenic bacteriophages make changes in bacterial genes expression, due to the ability of these phages to associate with the bacterial genome, especially if they integrated at an expressional attachment sites (Willey *et al.,* 2017). Some prophages contribute lysogenic conversion genes that are selective advantage to the bacterial host. Occasionally, phages are also involved in the lateral transfer of other mobile DNA elements or bacterial DNA (Canchaya *et al.,* 2008). Recent advances in the field of genomics have naked a major impact of phages in bacterial chromosome evolution.

Around 10% of bacteriophages in world are specifically targeting the members of the *Pseudomonas* genus. Traditionally, *Pseudomonas* bacteriophages had been used for the epidemiological discovering of specific strains; *e.g.,* the *Pseudomonas aeruginosa* typing

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phages of the Lindberg set and as tools in molecular biology (Ceyssens and Lavigne, 2010).

Material and Methods

Bacterial Strains Isolation

The study included obtaining 50 isolates of *P. aeruginosa* from different infection sources: burns, wounds, otitis media, UTI, diabetic foot and diarrhea at Salah-Addin hospital. The samples were identified according to the morphological and biochemical tests, the single pure colony of pale pinkish colonies (for lactose non-fermentable) on MacConkey agar media (HIMEDIA, India) were transferred to Cetrimide agar (nutrient agar from OXOID, England containing 0.3% of Cetrimide) to check if they were *P. aeroginosa* or not.

Bacteriophage Isolation and purification

Four samples of phages were collected from different sources by 500 ml containers: sewage sludge, which were obtained from Tigris river, road sewage already infected strains in Salah-Addin hospital and from cow, sheep feces in different places of Tikrit city; the samples were purified and experimented on the bacterial strains by plotting method and doubled agar layer by using Luria Bertoni Medium (LB: HIMEDIA, India) (Mohammed-Ali *et al.,* 2015; Zablocki *et al.,* 2016; Ferman and and Jameel, 2019).

Biochemical experiments

The bacterial strains were tested to detect some virulent factors before and after treating with bacteriophages, which included Blood lysis, gelatinase, alkaline protease, urease (Gupte, 2010), Biofilm formation by tube method on BHI (Fazlul *et al.,* 2018), Siderophore formation (Khalid 2019) and Beta lactamase (Naji 2016); these experiments were achieved on bacteria in order to evaluate their ability to produce these virulent factors before and after infecting with their bacteriophages.

Bacterial DNA isolation

A single colony was inoculated on 5 mL of nutrient broth and incubated over night at 37ºC to extract the total genomic DNA. Second day, 1.5 mL of a saturated culture was centrifuged for five minutes at 14,000 rpm. The pellet was re-suspended and lysed in 200 µL of "lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) by vigorous pipetting". A 66 µL of 5M NaCl solution was added and mixed well; then, the gluey mixture was centrifuged at 12,000 rpm for 10 minutes at 4ºC. The clear supernatant was transferred to a new Eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Followed by, centrifugation at 14,000 rpm for five minutes, the supernatant was removed to another Eppendorf tube and double volume of 100% ethanol was added. The tubes were gently inverted four to six times, then centrifuged at 10000 rpm for five minutes. The supernatant was discarded and 1 mL of ethanol (70%) was added to the pellet, and tubes were centrifuged at 10000 rpm for five minutes, this final step could be repeated to ensure clear DNA. Lastly, the supernatant was discarded and the pellet was dried for an hour at room temperature and then re-suspended in 100 µL of free nuclease water. The stock was kept at -20ºC until use. The DNA concentration was determined by measuring absorbance of the sample at 260 nm, using a spectrophotometer (Sambrok, 2001).

Detection Bacterial DNA variation by Specific PCR and RAPD-PCR

These tests were applied on bacteria in order to evaluate the ability of bacteriophages in making variation in bacterial DNA, so they achieved before and after infecting with their bacteriophages. The specific PCR detection included seven genes (*oprL, toxA, exoS, exoT, plcH, pslA and* bla_{IMP}) table 1 and their programs in

Table 1: primers that used in specific PCR.

No.	Primer Name		Sequence 5′→→→3′	Size (bp)	Reference
1.	*plcH*	F	GCACGTGGTCATCCTGATGC	608	Hilan (2019)
		R	TCCGTAGGCGTCGACGTAC		
2.	*pslA*	F	CACTGGACGTCTACTCCGACGATAT	1,119	Abdul Qadir (2015)
		R	GTTTCTTGATCTTGTGCAGGGTGTC		
3.	*exoS*	F	CTTGAAGGGACTCGACAAGG3	504	Fadhil *et al.,* (2016)
		R	TTCAGGTCCGCGTAGTGAAT		
4.	*exoT*	F	CAATCATCTCAGCAGAACCC	1,159	Finnan *et al.,* (2004)
		R	TGTCGTAGAGGATCTCCTG		
5.	*oprL*	F	ATG GAA ATG CTG AAA TTC GGC	500	Aljebory (2018)
		R	CTT CTT CAG CTC GAC GCG ACG		
6.	*toxA*	F	GGT AAC CAG CTC AGC CAC AT	352	Aljebory (2018)

Table 2: Specific-PCR programs.

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	cycles
plcH	94°C / 3min	94°C / 30sec	55°C / 60sec	72°C / 60sec	72°C / 5min	30
pslA	95°C / 5min	95°C / 60sec	55°C / 45sec	72°C / 75sec	72°C / 5min	30
exoT	96°C / 5min	94°C / 30sec	54°C / 30sec	72°C / 60sec	72°C / 5min	30
exoS - oprL - toxA	94°C / 4min	94°C / 30sec	55°C / 30sec	72°C / 45sec	72°C / 5min	30
blaIMP	94°C / 10min	94°C / 30sec	49.5°C /40sec	72°C / 50sec	72°C / 5min	36

table 2, while the RAPD-PCR included nine primers are mentioned in table 3 and their programs in table 4 according to Al-douri.

Table 3: RAPD-PCR primers.

No.	Primer	Sequence 5′→→→ 3′	No.	Primer	Sequence 5′→→→ 3′
1.	OPB-04	GGACTGGAGT	5.	OPC-08	TGGACCGGTG
2.	OPC-16	CACACTCCAG	6.	OPG-08	TCACGTCCAC
3.	OPB-12	CCTTGACGCA	7.	OPG-14	GGATGAGACC
4.	OPD-03	GTCGCCGTCA	8.	OPG-02	GGCACTGAGG
9.	OPY-04	GGCTGCAATG			

Table 4: RAPD-PCR program.

Stage	Temperature (time)	
Initial denaturation	94°C (4min)	
Denaturation	93°C (45sec)	40 cycles
Annealing	36°C (45sec)	
Extension	72°C (1.30min)	
Final extension	72°C (7min)	

Results and Discussion

Host Range

To know the host range of the isolated bacteriophages, spot test was applied for all bacterial strains and the infection percentage for each phage is demonstrated in table 5. The typing method is demonstrated in Fig. 1.

As its noticed in table 5, the maximum infection percent for the bacterial strains was (73%) by the phage

Table 5: Isolates Percentage That Were Infected with Their Phage.

Phages	Phage's source	Zone dimeter mm.	Isolates percentage that were infected with phage
P2	Tigris river	2.5	33.3%
P9	Sheep feces	1	73%
P11	Road sewage	2	60%
P15	Infected isolate	3	35%

5 (Ph9), while the minimum was by Ph2 with percent (33.3).

Bacterial Phenotypic Variation after Phages Infecting

It was noticed that several strains of bacteria were phenotypically changeable after infecting with bacteriophages. These changes in appearance were proved by virulent factors detection tests for *P. aeruginosa* before and after infection with bacteriophages, like blood lysis kind, biofilm formation, siderophore production, gelatinase, alkaline protease, urease and Beta-Lactamase.

All bacteriophages were experimented on bacteria and all of them proved their ability to make phenotypic variation in only four bacterial strains. The results showed that the production of biofilm, siderophore, alkaline protease and gelatinase was decreased in general; on the other hand, some strains after treating with phages became able to produce or lose certain enzymes; as its clear in table 6.

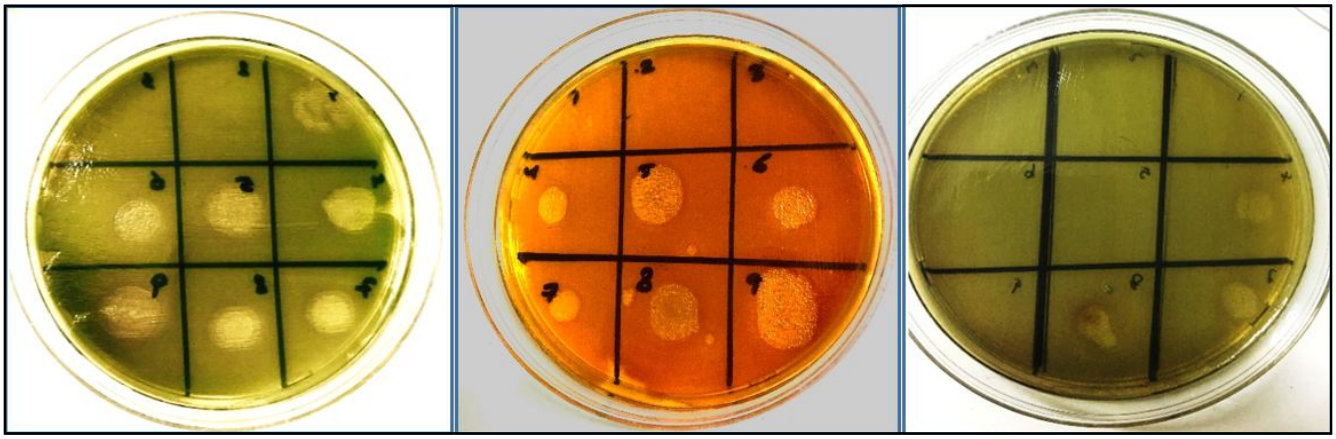

Fig. 1: Typing on solid agar.

Table 6: Enzyme production style in bacteria before and after treating with bacteriophages.

Enzyme type	Strain number	Phage type	Wild Production	Production after treating
urease	186	Ph11, Ph15	-	+
	230	P9	+	-
Beta-lactamase	181	Ph15	-	+
Hemolysis	22	Ph2	Alpha	Beta

Bacterial Specific PCR Before and After Treating with Bacteriophages

To ensure the effect of bacteriophages on bacterial expression, specific PCR test was applied on the same four strains that had clear phenotypic change in enzyme production after they were treated with their phages.

The seven tested primers were rechecked in four strains of bacteria after phage infection; the results showed that three primers (*opr*L, *exo*S and *psl*A) did not change in their results, while the remain four primers (*exoT*, *plcH*, *toxA* and *blaIMP*) results changed in the lysogenized bacteria. As it is shown in table 7, a *exoT* carrier strain was loosed it after infecting with Ph1, a non *plcH* and *toxA* gene carrier strains were acquired them after infected with Ph2 and Ph9 respectively, while the *toxA* gene was loosed after treating with Ph1. Finally, Ph15 enable one non carrier *blaIMP* strain to acquire it as PCR is displayed it in the Fig. 2.

From the previous result, it is clear that Ph1 was the most effective phage in specific PCR, as it made strain 240 loser of two genes: *exoT* and *toxA*.

Table 7: Genes detection by PCR in bacteria before and after treating with bacteriophages.

Gene kind	Strain number	Phage type	Wild Production	Production after treating
exoT	240	Ph1	+	-
plcH	22	Ph2	-	+
toxA	186	Ph9	-	-
	240	Ph1	+	-
blaIMP	181	Ph15	-	+

Bacterial RAPD Before and After Treating with Bacteriophage

OP B-04 Primer

This primer gave noticeable changes for bacteria; it showed disappearing and appearing of clear bands before and after treating with bacteriophages. The results are shown in table 8. The most obvious change in band number was in strain 186 which gave one band before treating with Ph9 and three bands after treating, while strain 240 gave one band before treating with Ph1 and four bands after treating. All produced bands after treating were in different sites from the old.

Table 8: The results of OP B-04 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	1	0	1
181	Ph15	0	1	1
186	Ph9	1	3	4
240	Ph1	1	4	5
Total	/	3	8	11

Total Variation % in bands for all samples by this primer: 3.67%.

OP C-16 Primer

Besides appearing and disappearing of band in bacteria by OP C-16 before and after treating with their bacteriophages, it should be mentioned that the strains 181 and 240 after infection with Ph15 and Ph1 respectively, they acquired two bands. The first band was at 600 bp whereas the second was close to 1400 bp with high copy number, as its clear in table 9.

OP B-12 Primer

This primer had been shown that Ph2 did not make

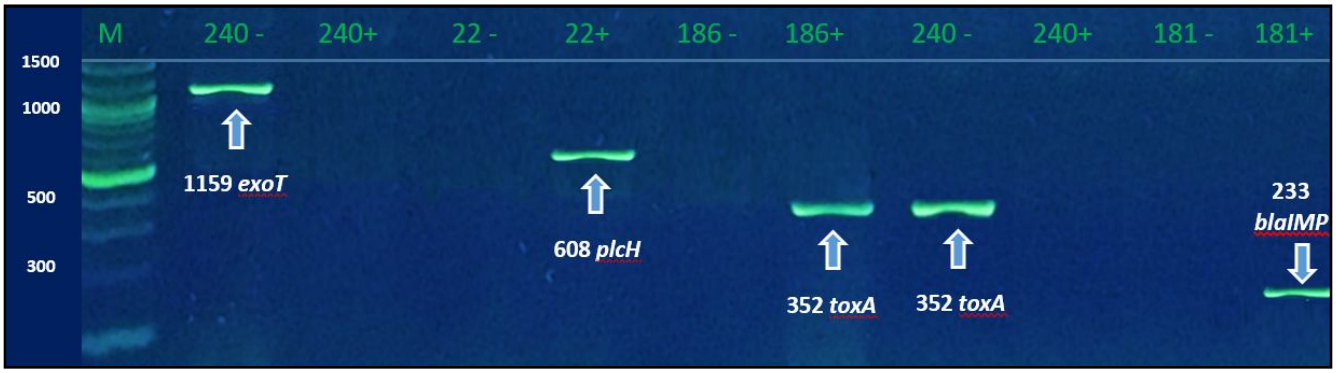

Fig. 2: Genes detection by PCR in bacteria before and after treating with bacteriophages. (-): the normal bacterial strain, (+): bacterial strain after treating with bacteriophage.

Table 9: The results of OP C-16 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	2	1	1
181	Ph15	1	2	3
186	Ph9	1	0	1
240	Ph1	0	2	2
Total	/	4	5	7

Total Variation % in bands for all samples by this primer: 1.75%.

any change in the genomic of the strain 22 except decreasing in the copy number of the band who had molecular weight more than 1500 bp; as table 10 showed, the same 5 bands appeared at their sites before and after infection with this phage. A main band at 330 bp was loosed in the bacteria 240 after it was infected with Ph1; appearing and disappearing of bands in the other strains also were shown by this primer.

Table 10: The results of OP B-12 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	5	5	0
181	Ph15	6	9	5
186	Ph9	4	7	5
240	Ph1	7	6	6
total	/	22	27	16

Total Variation % in bands for all samples by this primer: 0.73%.

OP D-03 Primer

All strains had differences in band number and their sites in appearing and disappearing before and after they were treated with their bacteriophage. It is so noticeable that two bands with high copy number were appeared at 600 and 490 bp for the strains: 181, 186 and 240 after they were lysogenized with their special phages, as its showed in table 11.

OP C-08 Primer

As previous RAPD-PCR tests, this reaction also showed the ability of phages to acquire or misplace their strains bands after infecting them. It should be mentioned that Ph15 was able to acquire the strain 181 three bands, whereas it had no one before infecting with it as its shown in table 12.

Table 11: The results of OP D-03 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	4	6	3
181	Ph15	5	4	5
186	Ph9	3	4	3
240	Ph1	3	2	5
total	/	15	16	16

Total Variation % in bands for all samples by this primer: 1.067%.

Table 12: The results of OP C-08 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	2	3	1
181	Ph15	0	3	3
186	Ph9	2	3	1
240	Ph1	3	4	3
total	/	7	13	8

Total Variation % in bands for all samples by this primer: 1.14%.

OP G-08 Primer

As its clear in table 13, the most affective strains were 181 by its phage Ph15, which showed difference in the number of band that appeared and missed before and after treating (10 bands); next was strain 240, which presented difference in the number of band that performed and missed before and after treating with Ph1 (7 bands).

OP G-14 Primer

This primer showed the high activity of Ph9, as it

Table 13: The results of OP G-08 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	5	3	2
181	Ph15	6	5	10
186	Ph9	3	5	4
240	Ph1	6	6	7
total	/	20	19	23

Total Variation % in bands for all samples by this primer: 1.15%.

made an obvious change after infecting the strain 186 by deleting and adding new primer binding sites to it; the sum of Appeared and disappeared bands were 11 bands as its clear in table 14.

Table 14: The results of OP G-14 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	6	5	1
181	Ph15	5	6	7
186	Ph9	6	7	11
240	Ph1	6	1	7
total	/	23	19	26

Total Variation % in bands for all samples by this primer: 1.13%.

OP G-02 Primer

Presence of many bands what characterized this primer. The test also detected that the produced bands after treating with phages were fewer than the original bands as clear in table 15, due to the activity of the phages to delete the primer binding sites on the bacterial strains which they infected.

Table 15: The results of OP G-02 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	7	10	7
181	Ph15	7	8	11
186	Ph9	9	6	5
240	Ph1	9	6	7
total	/	32	30	30

Total Variation % in bands for all samples by this primer: 0.94%.

OP Y-04 Primer

In general, this primer detected that the produced bands were more than the deleted by bacteriophages, it also showed that Ph9 was most active phage, it added 6 primer binding sites at the same time deleted 4 from the strain 186 as its obvious in table 16.

According to the number of appeared and disappeared bands for all nine previous RAPD-PCR primers, it is noticeable that the less bacteriophage made variation in the bacterial genome was Ph2 that infected strain 22 which missed and gained 18 bands after infection with Ph2. Whereas the most one were Ph15 that infect

Table 16: he results of OP Y-04 for bacteria before and after treating with phages.

Strain number	Phage type	Wild number of produced bands	Number of produced bands after treating	Appeared and disappeared bands
22	Ph2	3	5	2
181	Ph15	1	5	4
186	Ph9	7	5	10
240	Ph1	6	5	5
total	/	17	15	21

Total Variation % in bands for all samples by this primer: 1.24%.

strain 181 and was responsible for alteration about 49 primer binding sites at strain 181 genome, as its clear in table 17.

Table 17: The results of Entirety appeared and disappeared bands in bacteria by 9 primers after treating them with phages.

Strain number	Phage type	Entirety of appeared and disappeared bands for 9 primers
22	Ph2	18
181	Ph15	49
186	Ph9	45
240	Ph1	47

Probably, lysogenic bacteriophage makes changes in bacterial genes expression, due to the ability of these phages to associate with the bacterial genome, especially if they integrated at an expressional attachment sites as it had been noticed in specific PCR (Willey *et al.,* 2017) . Some prophages contribute lysogenic conversion genes that are selective advantage to the bacterial host (Canchaya *et al.,* 2008).

Prophages often encode 'morons' that are not directly involved in viral replication and can confer a benefit to their bacterial host. Such genes are independent transcriptional units of DNA and expressed at the same time as the phage is in the prophage state. Morons can include genes that enhance the virulence of their bacterial host, either directly (*e.g.* phage-encoded toxins) or indirectly, by enhancing the ecological fitness of bacteria during infection. That what had been shown in specific PCR of this study, when Ph9 enabled a *toxA* looser strain (strain 186) to possess this gene after infecting with Ph9. The effect of lysogenic conversion can be also seen clearly in the disease caused by cholera toxin; *V. cholerae* did not always cause disease, but the infected with the CTX phage gives the bacterium its toxinogenicity (Mettenleiter *et al.,* 2019; Davies *et al.,* 2016).

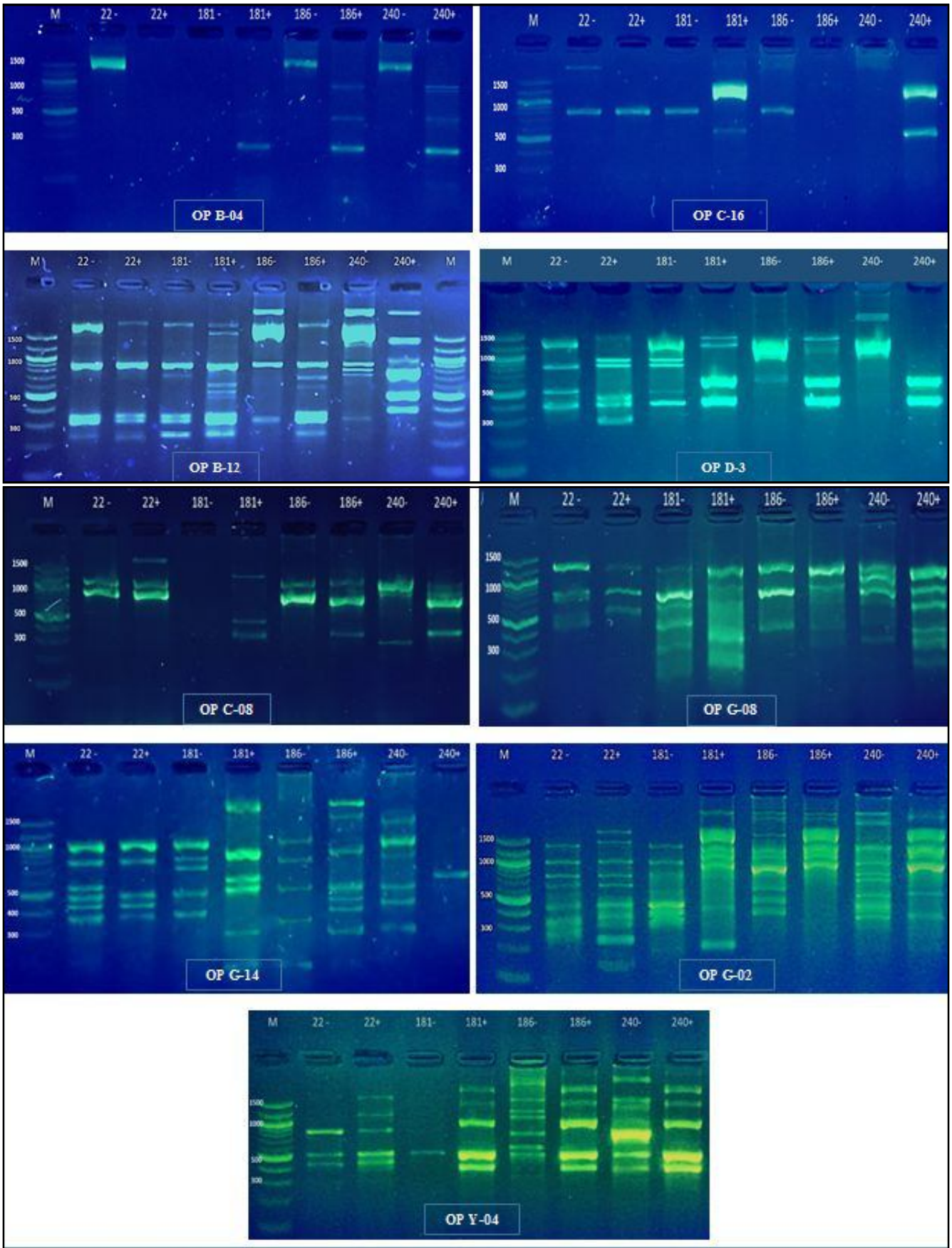

Figure 3: RAPD-PCR results for four bacterial strains before (-) and after (+) treating with four different phages.

Besides virulent genes, bacteriophages can package antibiotic resistance genes (ARGs) by transduction, contributing in rapid dissemination of resistances among bacteria especially penicillin's resistance genes (Gómez-Gómez *et al.,* 2019). That was obvious at bacterial phenotypic and genotypic behavior; in the manual experiment and specific PCR reaction the strain 181 became beta-lactamase positive and acquired bla_{IMP} after infecting with Ph15 (Gómez-Gómez *et al.,* 2019; Tang *et al.,* 2018).

Although many previous studies focused on the ability of lytic phage to decrease biofilm and showed that the lysogenic phages have only the ability to increase its production (Fortier and Sekulovic, 2013; Gómez-Gómez *et al.,* 2019); morphologically, this study showed the ability of lysogenic phages to reduce biofilm production. The decreasing of biofilm, siderophore and the other enzymes may cause by the affecting of these phages on bacterial genomic at all.

Disappearing or presence new bands in RAPD-PCR means making differences in the genome, which might due to insertion, deletion and substitution by the bacteriophages in bacterial genome where the sites of primer binding are existent by transduction; that what causes alteration in the arrangement of the remain nucleotides and then, the distance between two primers binding sites. That what prevents primer attachment at its specialized site on the genome at all, or creates new sites for binding (Williams *et al.,* 1990; Menouni *et al.,* 2015).

As its clear in Fig 3, some phages made narrow effect on bacterial genome; for example, when they tested by primer (OP C-16 and OP C-08), limited bands were changed after treating with bacteriophages. On the other hand, wide effect had been appeared by the other primers, many band renewed within different size and numbers. This alteration might cause by bacterial genome remodeling through bacteriophage specific-site recombination (Menouni *et al.,* 2015), it might also due to the ability of the other prophages to integrate and transpose randomly in genomes using DDE transposases as *Mu* phages do (Bobay *et al.,* 2013).

Conclusions

The four different distinguished types of lysogenic phages appeared ability in changing bacterial phenotypic and genotypic behavior, which revealed the ability of bacteria in evolution by acquiring and missing genes by these phages; that transduction was clear in both biochemical tests and molecular detection: (specific PCR and RAPD-PCR).

References

Al-douri, A.A. (2020). Evaluation the Molecular Effects of Ndemium and Diode Laser Types on Environmental and Clinical Isolates of *Pseudomonas aeruginosa*. MSc., Thesis. College of Science/Physics Department, Tikrit University.

Bobay, L., E.P. Rocha and M. Touchon1 (2013). The Adaptation of Temperate Bacteriophages to Their Host Genomes. *Mol. Biol. Evol*., **30(4):** 737-751.

Clokie, M.R. and A.M. Kropinski (eds.). (2009). Bacteriophages: Methods and Protocols. *Humana Press, a part of Springer Science+Business Media,* **1(501):** 267-286.

Canchaya, C., G. Fournous, S. Chibani-Chennoufi, M. Dillmann and H. Brüssow (2003). Phage as agents of lateral gene transfer. *Elsevier,* **6(I4):** 417-424.

Ceyssens, P. and R. Lavigne (2010). Bacteriophages of *Pseudomonas*. *Future Microbiol.,* **5(7):** 1041-1055.

Davies, E., C. Winstanley, J. Fothergill and C. James (2016). The role of temperate bacteriophages in bacterial infection. *FEMS Microbiology Letters*, **363(5):** 1-9.

Ferman, F.H. and Z.J. and Jameel (2019). Isolation and Identification Bacteriophages of Pseusdomonas aeruginosa Caused Wound and Burn Infections and Study of Antmicrobial Effective. *World Journal of Pharmacy and Pharmaceutical Sciences,* **8(5):** 129-144.

Fazlul, M.K., A. Najnin, Y. Farzana, M.A. Rashid, S. Deepthi, C.Srikumar, S.S. Rashid and M.H. Nazmul (2018). Detection of virulence factors and â lactamase encoding genes among the clinical isolates of Pseudomonas aeruginosa. *Journal of International Pharmaceutical Research,* **45:** 190-202.

Fortier, L. and O. Sekulovic (2013). Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence,* **4(5):** 354-365.

Gómez-Gómez, C., P. Blanco-Picazo, M. Brown-Jaque, P. Quirós, L. Rodríguez-Rubio, M. Cerdà-Cuellar and M. Muniesa (2019). Infectious phage particles packaging antibiotic resistance genes found in meat products and chicken feces. *Nature, Scientific Reports,* **9(1):** 1-11.

Gupte, S. (2010). The Short Textbook of Medical Microbiology (including parasitology) 10th ed., Jaypee Brothers Medical Publishers(P) Ltd, New Delhi, pp.476.

Howard-Varona, C., K.R. Hargreaves, S.T. Abedon and M.B. Sullivan (2017). Lysogeny in nature: mechanisms, impact and ecology of temperate phages. *The ISME Journal*, **11:** 1511-1520.

Kelly, C.M. and R. Heidi (2018). Microbiology: a systems approach. 5th ed. McGraw-Hill Education, 2 Penn Plaza, New York, NY.

Khalid, S.A. (2019). Determination of the *in-Vivo* and in vitro effectiveness of some plant extracts on Staphylococcus aureus isolated from wound infection. Ph.D. College of science, University of Tikrit.

Menouni, R., G. Hutinet2, M. Petit and M. Ansaldi (2015). Bacterial genome remodeling through bacteriophage recombination. *FEMS Microbiology Letters*, **362(1):** 1-10.

Mettenleiter, T., M. Kielian and M. Roossinck (2019). Advances in Virus Research. Academic Press, P:11-18.

Mohammed-Ali, M.N. and N.M. Jamalludeen (2015). Isolation and Characterization of Bacteriophage against Methicillin Resistant Staphylococcus aureus. *J. Med. Microb. Diagn.,* **5(1):** 213-218.

Naji, S.A. (2016). Antimicrobial Resistance Pattern, Biofilm Formation and Presence of *icaAD* Gene in Clinical Isolates of *Staphylococcus aureus*. M.Sc. Thesis. College of Science/ Biology Department, Tikrit University.

Ryan, K.J. and C.G. Ray (2014). Sherris Medical Microbiology. 6th ed., McGraw-Hill Education. P.617-623.

Riedel, S., J.A. Hobden, S. Miller, S.A. Morse, T.A. Mietzner, B. Detrick, T.G. Mitchell, J.A. Sakanari, P. Hotez and R. Mejia (2019). Jawetz, Melnick and Adelberg's Medical Microbiology, 28th ed. McGraw-Hill Education.

Sambrok, J.A. (2001). Molecular Cloning: A Laboratory Manual.

Tang, C., C. Deng, Y. Zhang, C. Xiao, J. Wang, X. Rao, F. Hu and S. Lu (2018). Characterization and Genomic Analyses of Pseudomonas aeruginosa Podovirus TC6: Establishment of Genus Pa11virus. *Front. Microbiol*., **9(2561):** 1-11.

Török, M.E., E.D. Moran and F.J. Cooke (2017). Oxford Handbook of Infectious Diseases and Microbiology. 2nd ed., Great Clarendon Street, Oxford, OX2 6DP, UK.

Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Rese*., **18(22):** 6531-6535.

Willey, J.M., L.M. Sherwood and C.J. Woolverton (2017). Prescott's Microbiology. McGraw-Hill Companies, New York.

Zablocki, O., E.M. Adriaenssens and D. Cowan (2016). Diversity and ecology of viruses in hyperarid desert soils. *Appl. Environ. Microbiol.,* **82:** 770-777.