Eco. Env. & Cons. 26 (August Suppl. Issue) : 2020; pp. (S9-S17)
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ISSN 0971–765X

The relationship between gene expression and Biodegradation of clorspan and Ground up pesticides by *Pseudomonas aeruginosa* bacteria

Beadaa Abdalqader Mahdii[1, 2] and Ahmed Mohammed Turke[1]

[1]Department of Biology /College of Science/University of Anbar
[2]Department of Biology /College of Science/University of Baghdad, Iraq

(Received 2 January, 2020; accepted 10 February, 2020)

ABSTRACT

18 bacterial isolates were isolated from 23 soil samples polluted with different pesticides, from Baghdad districts on both sides of Karkh and Rusafa. Where two PCR and qPCR tests performed, the results of the qPCR test for the 18 bacterial isolates of *P. aeruginosa* bacteria showed that the highest recorded gene frequency value in the biological treatment of Clorspan (Chlorpyrifos) recorded by bacterial isolation (5) where it recorded (356666382.4), and the highest value of genetic frequency in the biological treatment of Ground up (glyphosate) pesticide recorded by bacterial isolation (3) and reached to (52498.9155). As for the results of the PCR test showed genetic differences in the bacterial strains among *P. aeruginosa* isolation, and that the highest similarity raterecorded by the isolates (8,3), it reached to 89%.

Key words : *Gene expression, Pseudomonas aeruginosa, Clorspan*

Introduction

In the last decade and as a result of the increasing number of pollutants in wastewater, surface water, unground water and soil, much attention must be paid to dangerous chemical products such as pesticides which exist in the soil that can have various significant impacts which effects on human health (Teng *et al.,* 2010). These pollutants have hardly any biodegradability and resistance to treatment to traditional technologies (Bandala *et al.,* 2007). Microorganisms are used in the biological treatment of environmental pollutants (Singh, 2009; Ali, 2011). The use of microorganisms is very effective and natural process when compared with other methods that apply on biological treatment in pollutedsite (Sassman *et al.,* 2004, Nawab *et al.,* 2003).

The aim of the research

Finding the relationship between gene expression and biodegradations of Clorspan and Ground Up pesticides through the most efficient bacterial isolation of *Pseudomonas aeruginosa* bacteria.

Materials and Methods

Samples were collected during January and February 2019 by 23 samples from 6 stations (agricultural nurseries) lies in Baghdad city, which included the following areas (Amiriyah, Sayidiyah, Yarmouk, Naffak Al-shurtah, Palestine Street, Adhamiya) where these areas were polluted with different pesticides As a result of using them for agricultural purposes with different and unknown concentrations, Isolation of *Pseudomonas* bacteria from these

pollutes sites where a total of 18 bacterial isolates belonging to the *Pseudomonas aeruginosa* family isolated, later these types were diagnosed according to their ability to grow in MSM medium that contain pesticides at of 100 mg/L concentration (Patel and Desai, 1997), samples randomly collected from the soil surface layer (5-10 cm in depth) by using presterilized augar and transferred to sterile polyethylene bags, which were planted immediately after being brought to the laboratory at five samples per day.

Bacterial diagnosis

Bacteria diagnosed by external shape and electron microscope, and Biochemical tests, bacterial isolation detection by the VITEK2 device system (Pincus, 2013).

Examine PCR and qPCR

The materials listed in the table below used for the purpose of extracting the bacterial DNA, RNA and its purity measured according to Maniatis *et al.,* (1982).

The PCR interaction performed according to the conditions mentioned in (Palloma *et al.,* 2009).

The results of Real-Time (PCR) examination analyzed by the inflation curve. Amplification plot which depend on Throushold cycler number value (CT) where the sample becomes positive when it crosses the threshold line) Livakans Schmittgen, 2001), and as it shown in Equations below:

ΔCt = Ct of tested gene – Ct of housekeeping gene……..1

ΔΔCt = Ct (sample) – Ct (calibrator) ……2

Fold changes = 2-ΔΔCt…….3

Whereas:

Sample: is the target gene and housekeeping gene which are treated with pesticides.

Calibrator: It is the target gene for the target gene and the original reference gene for housekeeping gene which are not treated with pesticides.

The melting curve was within a temperature range of 60 °C - 90 °C with a temperature increase of 1 °C for every second.

Results and Discussion

Gene expression

The quantitative sequence polymerase interaction of bacterial isolates qPCR

The quantitative sequence polymerase interaction

Table 1. Shows the materials and kicks which were used in the research:

id	Equipment or material	Company (origin)
1	Thermocycler	Biorad T100 (USA)
2	Gel documentation	Cole Parmer (USA)
3	Luna Universal qPCR Master Mix	New England Biolabs (USA)
4	OneTaq Quick-Load 2X Master Mix with Standard Buffer	New England Biolabs (USA)
5	GENEzolTriRNA purification	Geneaid (Taiwan)
6	HiSenScript™ RH(-) RT PreMix	INTron (Korea)
7	Diamond dye	Promega (USA)
8	Agarose	ThermoFisher Scientific (USA)
9	Primers	New England Biolabs (USA)
10	Open qPCR	Chai (USA)
11	Free-nuclease water	New England Biolabs (USA)
12	**10X TAE buffer**	**Promega (USA)**

Primer selection

Table 2. Refers to the sequence or following up of the primwr which used to amplify PCR

Primer	Sequence (5' to 3')	References
16s rDNA	F- ACCTGGACTGATACTGACACTGA R- GTGGACTACCAGGGTATCTAATCCT	Goldsworthy, 2008
opd	F- TCACACTGACTCACGAGC R- CGGCCAATAAACTGACGT	Kwak *et al.,* 2012

F: Forward R: Reverse

Table 3. Refers to Quantitative chain polymerase interactionqPCR:

No.	Stages	Temperature	time	No. of Cycle
1	Reverse transcription	42	15 minutes	1cycle
2	Enzyme inactivation	95	10 minutes	1cycle
3	Denaturation	95	30 seconds	40 cycles
4	Annealing	59°C	30 seconds	
5	Extension	72ºC	30 seconds	

qPCR technique is a modern, fast, sensitive and highly specific technique. This technique used to detect diseases in humans and animals in some studies (Ward and Bej, 2006) it contributes to monitoring epidemic diseases, including bacterial species contamination and in typical time. High accuracy, free from confusion and skepticism, and thus contribute to improving public health (Aridgides *et al.*, 2004; Bauer and Rorvik, 2007).

The results of the quantitive sequence polymerase interaction qPCR analyzed by the Amplification Curve based on the Threshold cycler number (CT) value where the sample is positive when it crosses threshold line, and the specificity of the examination for positive samples determined by the melt Curve where the samples are positive if they appear at the same melting degree (Tm), which ranges in the actual inflation of the gene between 60 °C- 90 °C.

Through the results of the current study, And according to Table 4 and 5, which showed a large variation in the positive test for quantitative sequence polymerase interaction qPCR of bacterial isolates *P. aeruginosa* (18) under study during treatment process (Clorspan and Ground-up) pesticides, Where bacterial isolates (1, 2, 3, 4, 5, 6, 7, 8, 9A, 9B, 13, 14, 15, 16, 17, 34, B) respectively showed positive result in quantitative sequence polymerase interaction examination qPCR, isolation (5) Came and the recorded value was (356666382.4) as the highest isolate that showed the highest gene frequency,And for bacterial isolation (18), it showed the negativity of quantitative sequence polymerase interaction examination qPCR during the treatment with Clorspan pesticide and the recorded value was (0.358488812), so if the gene frequency is higher than <1, it indicates positive isolation as well as the significance of isolation. For bacterial isolates treated with Ground up pesticide, isolates showed (1,2,3,4,5,6, B), respectively, and positive tests in the

Table 4. Shows the results of quantitative sequence polymeraseinteraction qPCR examination of the bacterial isolates *P. aeruginosa* (18) before and after treatment with Clorspan for (16SrRNA) and (opd) genes.

	before treatment			after treatment clo				
isolate	16SrRNA	opd	ΔCt	16SrRNA	opd	ΔCt	ΔΔCt	fold change
1	12.24	28.31	16.07	33.68	35.29	1.61	-14.46	22536.87715
2	10.76	29.52	18.76	30.88	25.08	-5.8	-24.56	24734133.1
3	11.2	24.31	13.11	30.3	33.27	2.97	-10.14	1128.350839
4	11.74	25.58	13.84	33.48	33.1	-0.38	-14.22	19082.99508
5	12.13	33.16	21.03	32.08	24.7	-7.38	-28.41	356666382.4
6	10.44	31.72	21.28	33.65	40	6.35	-14.93	31216.04192
7	9.83	28.42	18.59	32.66	33.14	0.48	-18.11	282913.2068
8	11.72	29.03	17.31	32.51	33.94	1.43	-15.88	60305.41787
9A	20.66	28.39	7.73	30.89	30.71	-0.18	-7.91	240.5178238
9B	11.65	32.17	20.52	33.48	30.93	-2.55	-23.07	8805661.77
13	21.45	30.48	9.03	32.43	32.37	-0.06	-9.09	544.9575334
14	22.1	32.75	10.65	31.93	32.98	1.05	-9.6	776.0468821
15	22.1	28.91	6.81	32.54	31.37	-1.17	-7.98	252.4755724
16	22.09	27.07	4.98	31.09	30.52	-0.57	-5.55	46.85074227
17	21.45	28.93	7.48	33.84	30.98	-2.86	-10.34	1296.134752
18	21.84	30.28	8.44	21.6	31.52	9.92	1.48	0.358488812
34	21.21	30.69	9.48	23.62	31.58	7.96	-1.52	2.867910496
B	11.57	32.49	20.92	18.98	32.27	13.29	-7.63	198.0883192

qPCR and isolation (3) showed the highest gene frequency values which was (52498.9155), As for bacterial isolates (7, 8, 9A, 9B, 13, 14, 15, 16, 17, 18, 34), it showed negative examination and isolation (16) recorded lowest value and it was (0.01074642), the negativity of the genetic frequency examination does not mean in efficiency of the isolates in treatment process or biocracker of pesticides, as the rest of the other isolates showed a positive genetic frequency test, which is from the same family belonging to *P. aeruginosa* bacteria, which may have these bacterial groups that showed another negative mechanical examination in the biodegradation process of the pesticides. The results of the current study of *P. aeruginosa* bacteria (18) bacterial isolates were confirmed positive and negative isolates also according to the appendices shown for Amplification Curve and melt Curve fusion curves for genes (16SrRNA) and (opd) in (1), (2) and (3)) And (4) Appendices, respectively.

In order to apply a quantitative sequence polymerase interaction to evaluate the efficacy of the biodegradation or biodegradations of OPH (Oreganophosphorus hydrolase), the opd gene identified, as the gene that was responsible for Hydrolysis process of ester bond in organic phosphorous compounds, so the opd gene was identified as a target gene In this study. The appropriate primer for this gene determined and designed by depending the access number or sequence number (accession no.) And its match with the opd gene for *P. aeruginosa* bacteria, as well as the primer that belong to 16SrRNA gene (Fierer *et al.*, 2005). The efficacy of the Biocracking process of (clorspan and Ground-up) pesticide verified by *P. aeruginosa* bacteria by using quantitive sequence polymerase interaction qPCR technique through the target opd gene, as the

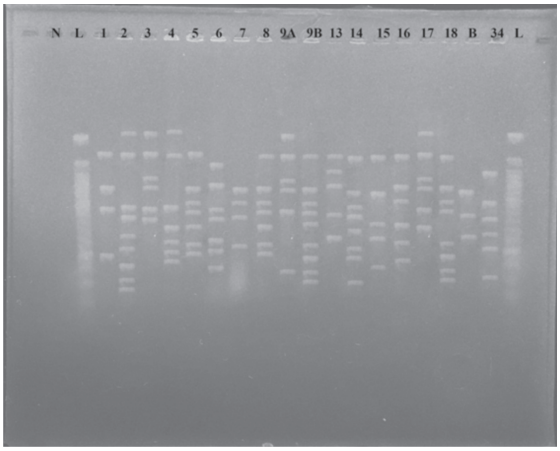


Fig. 1. Shows the genetic fingerprint in sequence polymerase interaction(PCR fingerprinting) of P. aeruginosa bacterial isolates where L-Ladder represents the sequence from 1-34 *P. aeruginosa* bacterial isolates.

Table 5. Shows the results of quantitative sequence polymerase interaction qPCR examination ofbacterial isolates *P. aeruginosa* (18) before and after treatment with Ground up for (16SrRNA) and (opd) genes.

isolate	before treatment			after treatment G				
	16SrRNA	opd	ΔCt	16SrRNA	opd	ΔCt	ΔΔCt	fold change
1	12.24	28.31	16.07	16.29	31.05	14.76	-1.31	2.479415
2	10.76	29.52	18.76	14.33	30.61	16.28	-2.48	5.578975
3	11.2	40	28.8	15.74	28.86	13.12	-15.68	52498.92
4	11.74	25.58	13.84	16.53	30.04	13.51	-0.33	1.257013
5	12.13	33.16	21.03	13.71	29.73	16.02	-5.01	32.22258
6	10.44	31.72	21.28	9.95	30.29	20.34	-0.94	1.918528
7	9.83	28.42	18.59	11.86	32.13	20.27	1.68	0.312083
8	11.72	29.03	17.31	8.8	31.66	22.86	5.55	0.021344
9A	20.66	28.39	7.73	22.58	31.55	8.97	1.24	0.423373
9B	11.65	32.17	20.52	12.93	33.76	20.83	0.31	0.806642
13	21.45	30.48	9.03	20.91	32.59	11.68	2.65	0.15932
14	22.1	32.75	10.65	20.45	32.29	11.84	1.19	0.438303
15	22.1	28.91	6.81	20.94	32.45	11.51	4.7	0.038473
16	22.09	27.07	4.98	21.02	32.54	11.52	6.54	0.010746
17	21.45	28.93	7.48	20.12	33.49	13.37	5.89	0.016863
18	21.84	30.28	8.44	21.68	32.43	10.75	2.31	0.20166
34	21.21	30.69	9.48	22.04	32.49	10.45	0.97	0.510506
B	11.57	32.49	20.92	9.97	29.73	19.76	-1.16	2.234574

high ability to break pesticides (organic phosphorous) indicates the high effectiveness of the opd gene Through the ability to produce OPH analyzers (Singh and Walker, 2006). This was also confirmed by the experiment in which the remaining pesticide concentrations which measured and treated by *P. aeruginosa* bacteria and measured by the GC ppm device where positive isolates showed their efficiency in the biodegradation process of pesticides, as shown in Table 2-3 and also (Kwak *et al.*, 2012) indicate in their experience the efficacy of the opd gene in pesticide biocracking in a miniature soil environment of Tolclofos-methyl pesticide, (O, O-dimethyl O- (2,6-dichloro-4-methylphenyl) phosphorothioate)), as the opd gene proved it efficiency in the biodegradation process of the pesticide which used in the experiment by measuring the gene frequency by the researcher.

2-Genotyping

(RAPD –PCR) (Random Amplification of Polymorphic DNA) performed to distinguish accurately between the 18 isolates *P. aeruginosa* divided to six groups according to similarity among them, the first group included isolates (1,14,17, 18 B) and the similarity rate of 53%, and the second group included isolates (14,17, 16, B) with a similarity rate of 59%, while the third group included isolates (34, 5, 14, 17,18) and the similarity rate was 66%, the fourth group included isolates (3, 8, B9) with a similarity rate of 85%, and the fifth group included isolates (3, 8) and the similarity rate was 89% as the highest similarity rate between isolates, for bacterial isolates (7, 15, 13, 2, 16, 6, A9, 4), these isolates were not combined with any of the groups above as shown in Fig. 1, 2.

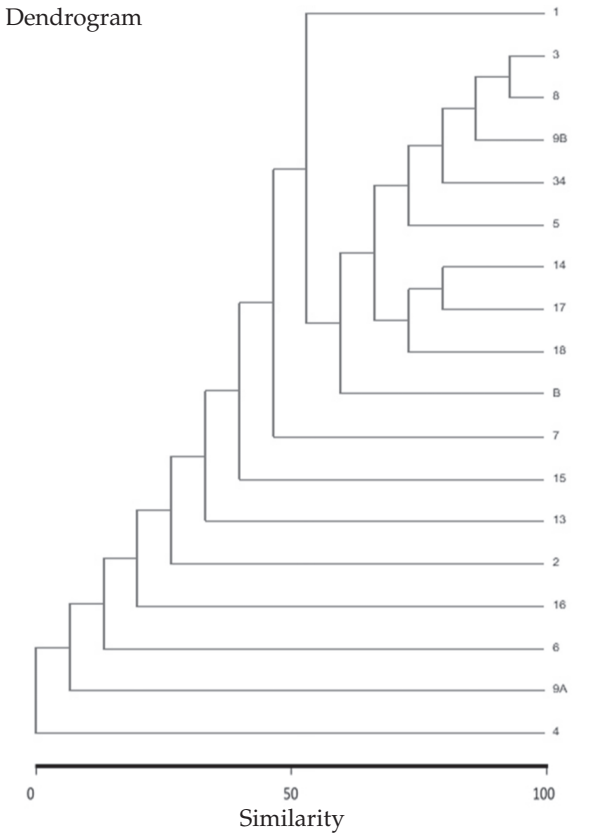
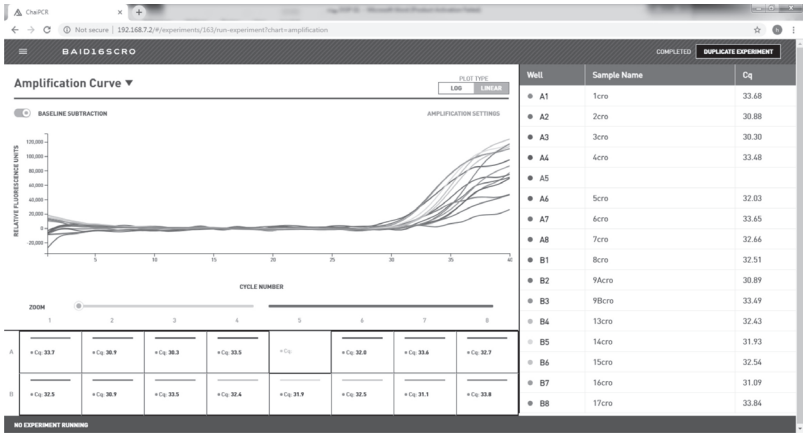


Fig. 2. Shows the genetic analysis of (Cluster analysis of genetic) groups using the DNA fingerprint patternof random amplification of polymorphic DNA (RAPD –PCR) for bacterial environmental isolates *P. aeruginosa*.

The RAPD-PCR fingerprint applied on the types of bacterial isolates *P. aeruginosa* and can be considered a useful complementary tool for environmen-



Appendix 1. Shows Amplification Curve for the *16SrRNA* gene for bacterial isolates *P. aeruginosa* during the qPCR.

Appendix 2. Illustrates Melt Curve for the *16SrRNA* gene for bacterial isolates *P. aeruginosa* during qPCR treatment with clorspan and ground-up pesticides.

Appendix 2. Illustrates AmplificationCurve for the *16SrRNA* gene for bacterial isolates *P. aeruginosa* during qPCR treatment with clorspan and ground-up pesticides.

Appendix 4. Shows the Amplifications Curve for the opd gene for bacterial isolates P. aeruginosa during qPCR treatment with clorspan and ground-up pesticides.

tal studies of groups of this species (Wolska *et al.*, 2012), and the result also shows differences in the genotype between the isolates. The genotype , used to distinguish between these bacterial chains based on their genetic content, recently become widely used to know bacterial chains, the methods used in the genotype of bacteria are completely different from each other (Yildirim *et al.*, 2011).

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