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Molecular study of the relationship of gene expression of some genes with the temperature variation of bacterial growth

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen responsible for serious infections. At least three different exopolysaccharides, alginate, polysaccharide synthesis locus (*Psl*), and pellicle exopolysaccharide (*Pel*) make up the biofilm matrix in *P. aeruginosa* . The effect of temperature on the biofilm formation and gene expression was examined by microtiter plate and real-time quantitative polymerase chain reaction (qRT-PCR). To be able to determine the effect of temperature on biofilm formation and gene expression of *P. aeruginosa*, 303 clinical and environmental samples were collected. *Pseudomonas aeruginosa* was isolated from 61 (20.1%) and 48 (15.8%) of the clinical and environmental samples, respectively. The ability of clinical and environmental *P. aeruginosa* isolates to develop biofilm was observed in 86.9% and 85.42% of the isolates, respectively, distributed into strong, moderate, and weak biofilm producers. Moreover, gene expression for *pslA*, *pelA* and *algD* genes was estimated for clinical and environmental isolates, the clinical *P. aeruginosa* isolates showed the highest biofilm production and the highest gene expression of *pslA*, *pelA* and *algD* genes as compared to environmental isolates when temperature changed. In summary, both clinical and environmental isolates formed biofilm and carried *psl A*, *pel A* and *alg D* genes regardless of the intensity of the biofilm. Also, 37°C represented the best temperature for biofilm production.

Keywords: *algD*, Biofilm, gene expression, *pela*, *pslA*, temperature.

Introduction

Pseudomonas spp. are found everywhere in the soil, a number of aqueous solutions, such as disinfectants, soaps, eye drops, as well as sinks and respiratory devices [1]. *Pseudomonas aeruginosa* is non-fermenting Gram-negative bacterium [2]. In humans, *P. aeruginosa* is an opportunistic pathogen responsible for serious infections[3]. It is the major bacterial pathogen that colonizes cystic fibrosis patients and is one of the most common infectious agents in nosocomial infections, along with severe burn, cancer, transplantation and acquired immunodeficiency syndrome(AIDS)[4].Also in the hospital, it is responsible for the formation of biofilms on the medical device surfaces[5].Several virulence factors may cause its pathogenicity[6], one example is the presence of lipopolysaccharide (LPS).The LPS is the primary element of the outer surface of *P. aeruginosa* that creates biofilm. A

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Baghdad Science Journal

biofilm is a collection of microorganisms that are enclosed in extracellular polymeric substances, which are made up of proteins, polysaccharides, and extracellular DNA[7]. The function of biofilms is to ensure the protection of the microorganisms within them against the threat of the bacterial aggressive actions of both internal and external environment[8]. Additionally, due to limited diffusion inside biofilms, which limits the actual dose that reaches the bacterium, antibiotic treatment against human inflammation is ineffective or worthless. In other words, bacteria that form biofilms are 100–1000 times more resistant to antimicrobial chemicals[9]. The biofilm matrix in *P. aeruginosa* is composed of at least three distinct exopolysaccharides, alginate, polysaccharide synthesis locus (Psl), and pellicle exopolysaccharide (Pel) [10]. Production of numerous structurally distinct polysaccharides may enable cells to adapt to particular surroundings because the structure of a polysaccharide is crucial to its function. It has been demonstrated that the cohesive and adhesive qualities of alginate, *psl*, and *pel* enable cells to form pellicles, microcolonies, or biofilms [7]. Studies have shown that subjecting biofilms to multiple ecological factors, comprised of physical elements like temperature, light penetration, and water current, as well as chemical ones like pH, nutrient availability, and toxicant effects, can cause the expression of various gene sets, which in turn produce different biofilm morphologies[11]. A new study has demonstrated the presence of a relationship between temperature and biofilm development in opportunistic bacterial pathogens, like *P. aeruginosa*, and that correlation is controlled by cyclic di-GMP signaling [12]. Thus, this research aimed to determine the effect of temperature on biofilm formation and gene expression of *P. aeruginosa.*

Materials and Methods

Bacterial Isolation and Identification

A total of three hundred and three samples from clinical (148 samples) and environmental (155 samples) sources were collected in the current study, among 109 isolates identified as *P. aeruginosa* 61 isolates were clinical and 48 environmental isolates. The clinical samples were collected from patients referred to Al-Yarmouk hospital in Baghdad city, Iraq, from September 2021- February 2022. These samples included urine, wound infections, burns, and ear infection. The environmental samples represented Al-Yarmouk hospital wastewater; which was collected from the site located before the hospital treatment unit[13]. The isolates were subjected to standard microbiological testing procedures that included a variety of enrichment, differential and selective media(Nutrient agar, MacConky agar, and cetrimide agar), colony morphology examination by Gram's stain, as well as a variety of biochemical analyses(oxidase and catalase test) and VITEK 2 system were depended to complete the identification of *P. aeruginosa* isolates.

Biofilm Formation Assay

The ability of the *P. aeruginosa* isolates to form biofilm at different temperatures, was carried out in two steps: firstly, *P. aeruginosa* isolates were cultured overnight at three different temperatures 27, 37, and 47°C in tryptic soy broth containing 0.25 % glucose. The second step was conducted by using microtiter plate biofilm formation assay as described by Bahador *et al*. [14]. In this technique, broth cultures (from the first step) were tested using the same medium as a diluent compared to McFarland standard tube No. 0.5. A 96-well flat-bottomed polystyrene plate with three wells was inoculated with 125 µL of an isolate suspension each, then they were incubated at 37°C for 24 hours. Thereafter, all wells were washed three times with 300 µL of distilled water. The wells were dried in an inverted position at room temperature, and then stained for 10-15 min with 125 µL of a 0.1% crystal violet solution in water. Crystal violet was removed, and the wells were washed three times to remove any remaining crystal violet. With 125 µL of 30% of acetic acid, the wells were de-stained. A new sterile, 96-well polystyrene microtiter plate was inoculated with 125 µL of the de-staining solution. To measure the absorbance of the de-staining solution, an ELISA reader (Stat Fax-2100), was used and the absorbance was determined at 490 nm. Each test was carried out three times. The background OD was calculated using the un-

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Baghdad Science Journal

inoculated media as a control. Three standard deviations more than the mean OD of the negative control were designated as the cutoff OD (ODc). According to the readings of the microtiter plate, the isolates were divided into four categories: strong biofilm producers (4*ODc< ODi), moderate biofilm producers (2*ODc <ODi< 4*ODc), weak biofilm producers (ODc< ODi< 2*ODc), or non-producers of biofilm (ODi <ODc).

Expression of genes

In this study, the biofilm genes including *pslA*, *pelA* and *algD* of four clinical and environmental isolates *P. aeruginosa* were assayed for expression analysis. These isolates represented strong biofilm producers. *16 SrRNA* gene served as the reference gene.

A- RNA Extraction

Employing Trizol reagent and the manufacturer's instructions (Promega, USA), RNA was extracted from planktonic *P. aeruginosa* cells. The bacterial cells were harvested in a microcentrifuge tube by centrifuging for 1min at 13,000 × g, and this step was repeated to obtain enough amounts of cell pellets. The pellet was re-suspended in 0.5 mL of Trizol and incubated for 5 min to permit complete dissociation of the nucleoprotein complex. After that, 0.15 mL of chloroform was added according to thermos fisher instructions used for lysis and incubated for 2–3 min. The sample was centrifuged for 15 min at 12,000 × g and then, the mixture was separated into a lower red phenol-chloroform, interphase, and a colorless upper aqueous phase. The aqueous phase containing the whole cell RNA was transferred to a new tube, and the RNA was precipitated by adding 0.45 mL of isopropanol to the aqueous phase. After that, the mixture was incubated for 10 min and centrifuged for 10 min at 12,000 × g. At this step, the total RNA precipitate formed a white gel-like pellet at the bottom of the tube. The supernatant was discarded by micropipette and re-suspended by 0.75 mL of 75% ethanol. Then, the vortex was used to dissolve the pellet, which was centrifuged for 5 min at 7500 × g and the supernatant was discarded by micropipette. To dry the RNA pellet, the tube was opened for 15 min, the pellet re-suspended by 20 µl of RNase- free water, and incubated at 60°C for 15 min by using a thermos mixer. Total RNA samples were stored at -20°C until use.

B- RNA integrity and quality

This assay is for the determination of the purity and concentration of RNA. In this technique, Qubit™ RNA HS Assay Kit (Q32852) was used, the Qubit® working solution was prepared by diluting the Qubit® RNA HS Reagent 1:200 in Qubit® RNA HS buffer. After that, 190 µL from Qubit® working solution has been added to each tube designed to be as a standard, then 10 µl from each provided standard solution has been added into the same tubes, then vortexed. Then, 197 µL has been added to each tube prepared for the sample and then 3 µL of the sample was added individually. All components were vortexed and incubated at room temperature for 3 min. Finally, standard tubes were inserted into Qubit instrument for creating the concentration curve. Tubes for samples have been added one by one to read the concentration of RNA in each sample.

C- Reverse transcription reaction

The first step to study gene expression was a conversion of whole cell RNA to complementary DNA (cDNA) using protoscript cDNA synthesis kit (NEB, UK). The cDNA synthesis was performed by adding 5µL from each extracted total cell RNA into a new PCR tube. Then, 10 µL of protoscript reaction mix (containing dNTPs, buffer and other essential components) was added to each sample. After that, 2 µL of MuLV enzyme and 2 µL of oligo dT were added to each sample. Finally, the total volume was completed up to 20 µL by adding 1 µL of nuclease-free water. This mixture was incubated for 1 hour at 42ºC by using thermocycler, and this was followed by incubation at 80ºC for 10 min for inactivation of the enzyme reverse transcriptase.
The cDNA product was also quantified by using Qubit 4.0, and stored until performing the second step of qRT-PCR (Relative quantitative).

D- Quantitative real-time PCR

Gene expression was studied by qRT-PCR using bioer-Germany. The cDNA samples from clinical and environmental *P. aeruginosa* isolates were used. For each sample, there were four PCR tubes, one tube for each gene (*algD, pelA, pslA* and *16S rRNA*), with the last one being the housekeeping

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Baghdad Science Journal

gene of this study. The detection of quantity based on the use of SyberGreen. Table 1 lists the primers and their sequences used in the present study. Table 2, shows the qRT-PCT reaction mixture components with their amounts.

Table 1. Sequences of the primers employed in the study

Primers		Primer sequence (5''→3')	Product size	Reference
pslA	F	ATAAGATCAAGAAACGCGTGGA	146 bp	[15]
	R	TGTAGAGGTCGAACCACACCG		
PelA	F	CCTTCAGCCATCCGTTCTTCT	118 bp	
	R	TCGCGTACGAAGTCGACCTT		
Alg D	F	GAGGAATACCAGCTGATCCGG	129 bp	Designed in present study
	R	CACCGAGTTCAAGGACCTGAA		
Ps16SrRNA	F	ACCTGGACTGATACTGACACTGA		[16]
	R	GTGGACTACCAGGGTATCTAATCCT		

Table 2.Components utilized in the qRT-PCR in reaction mixture

Components	Amount
Universal qPCR Master Mix	10 µl
10 µM of Forward Primer	1 µl
10 µM of Reverse Primer	1 µl
Template cDNA	5 µl
Nuclease-Free Water	3µl
Total volume	20 µl

Prior to running qRT-PCR, the PCR tubes were spun for 1 min at 2000 xg to remove any bubbles. The cDNA samples from clinical and environmental isolates were included in the same PCR run. Table 3, illustrates the program of qRT-PCR.

Table 3. Protocol of qRT-PCR

Phase	Temp (°C)	Time	Cycles
1st Denaturation	95	60 sec	1
Denaturation	95	15 sec	45
Extension	60	30 sec +plate (read)	
Melt curve	60 - 95	40 min	1

E- Calculating gene expression

The results of qRT-PCR were analyzed according to Livak and Schmittgen formula. The difference in cycle threshold (ΔCt) and fold changes were evaluated between the treated groups and the calibrators of each gene[17]. These values were normalized to *16SrRNA* gene expression, as shown below:

$\Delta\Delta Ct = \Delta Ct \text{ (test samples)} - \Delta Ct \text{ (calibrator samples)}$

$\Delta Ct \text{ (test samples)} = Ct \text{ (target gene in test)} - Ct \text{ (reference genes in test)}$

$\Delta Ct \text{ (calibrator samples)} = Ct \text{ (target gene in calibrator)} - Ct \text{ (reference genes in calibrator)}$

$\text{Fold change in gene expression} = 2^{-\Delta\Delta Ct}$

Statistical analysis

The statistical package for social science (SPSS) 2018 was used to analyze the ability of clinical and environmental *P. aeruginosa* isolates to produce biofilm. Counts and percentages were used to form categorical data, t-test was utilized to assess how temperature affected the biofilm[18].

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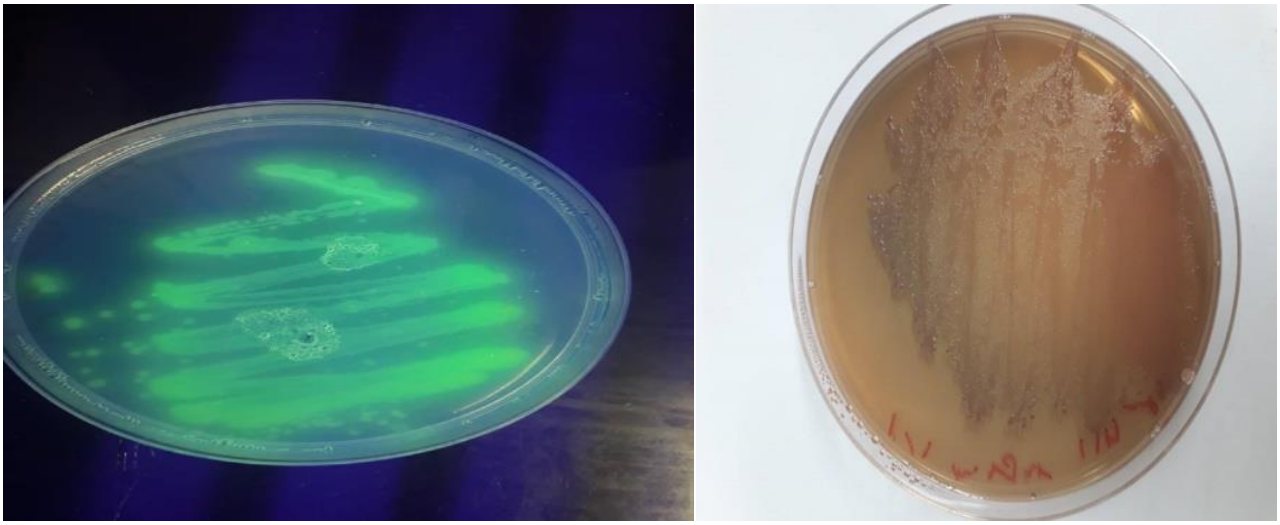
Baghdad Science Journal

Results and Discussion

Isolation and identification of *P. aeruginosa*

The results of all conventional identification tests revealed that, among 148 clinical samples, 61 isolates (41 %) were identified as *P. aeruginosa*. Whereas out of 155 environmental samples 48 (31%) isolates of *P. aeruginosa* were identified. Cultivation on Nutrient agar, MacConkey agar, and cultivated on Cetrimide agar was shown in Fig.1. Colonies on Cetrimide agar were characterized by a shiny green pigment due to the production of pyocyanin. Afterward, all *P. aeruginosa* isolates were characterized using biochemical testing for confirmatory identification.



A B

Figure 1. *P. aeruginosa* colonies on (A) Cetrimide agar and (B) MacConkey agar, after 24 hours of incubation at 37ºC.

Microscopically, the study showed that these isolates belonged to *Pseudomonas* spp. which appeared as Gram-negative rods, non-spore forming which initially indicated as *P. aeruginosa* in agreement with Markey *et al*,[19]. Microscopic examination for target isolates was carried out using Gram stain, so the 61 isolates of clinical and 48 of environmental origins were often stained with pink gram referred to as negative gram, and straight or slightly rod shapes appeared. These isolates showed positive results for oxidase and catalase and were capable of producing catalase which reduces hydrogen peroxide (H_2O_2) to water and oxygen gas bubbles. Cetrimide agar is recognized to be toxic and inhibit other microbial flora. Cetrimide agar used to cultivated *P. aeruginosa*, and all *Pseudomonas* isolates were able to grow at 42ºC. At the same time, no growth was noticed at 4ºC[20] as shown in Table 4. Finally, the identification of *P. aeruginosa* isolates was confirmed by using VITEK 2 compact system, the result from VITEK 2 compact system was in agreement with those obtained from biochemical identifications, that confirmed the isolates belonged to clinical and environmental *P. aeruginosa* were 61 (41%) and 48 (31%) isolates, respectively as shown in Fig. 2.

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Baghdad Science Journal

Table 4. Biochemical tests for *Pseudomonas aeruginosa*

Test	Results
MacConkey agar medium	Non-lactose fermented Pale elevated colonies
Cetrimide agar	+growth with fluorescent green color, elevated colonies, and grape-like odor
Oxidase test	+purple color
Catalase test	+ Bubbles
Pigment production	+ pigments
Growth at 42^{0}C	**+**
Growth at 4^{0}C	**-**
(+)Positive, (-) Negative	

bioMérieux Customer: Microbiology Chart Report Printed February 13, 2022 8:22:25 AM AST

Patient Name: Ahmed Attalla Hasan
Location:
Lab ID: 141
Patient ID: XCVXCB CX
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	8.00 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Pseudomonas aeruginosa** 0003051103500352		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	(-)	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	+	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ILLM	-	64	ILATa	+			

Figure 2. Chart Report of VITEK-2 System for identification of *Pseudomonas aeruginosa.*

Ability to produce biofilms

The capacity of *P. aeruginosa* to produce biofilms is presented in Fig. 3 and 4 which revealed that from 61 clinical isolates, 53 (86.8%) of *P. aeruginosa*, were biofilm producers, distributed into 25 (47.2%) as strong biofilm producers, 13 (24.5%) moderate producers, 15 (28.3%) weak producers, and the non-biofilm producers were 8 (13.11%). On the other hand, the present findings indicated that of the 48 *P. aeruginosa* retrieved from the environmental isolates 41 (85.42%) were identified as biofilm producers, involving 13 (31.71%) possessed the strong biofilm-forming ability, 11 (26.83%) had the moderate ability and 17 (41.46%) exhibited weak ability, and the non-biofilm producers were 7 (14.58%).

2024, 21(2): 0313-0327
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Baghdad Science Journal

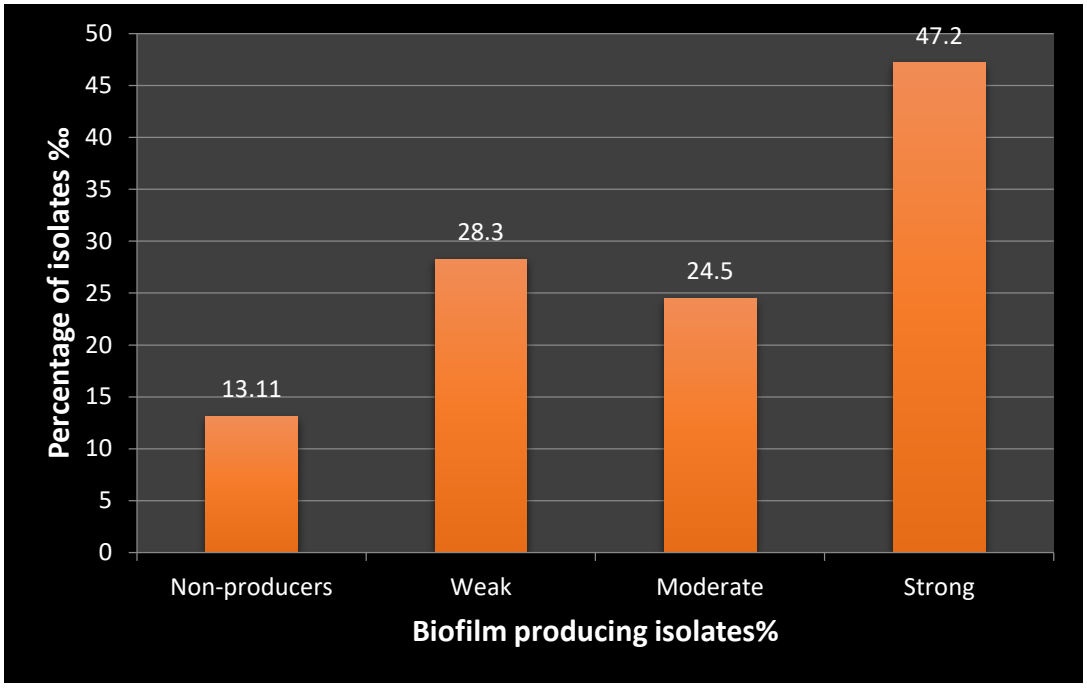

Figure 3. Distribution of clinical *P. aeruginosa* isolates, into non-producers, weak, moderate and strong biofilm producers.

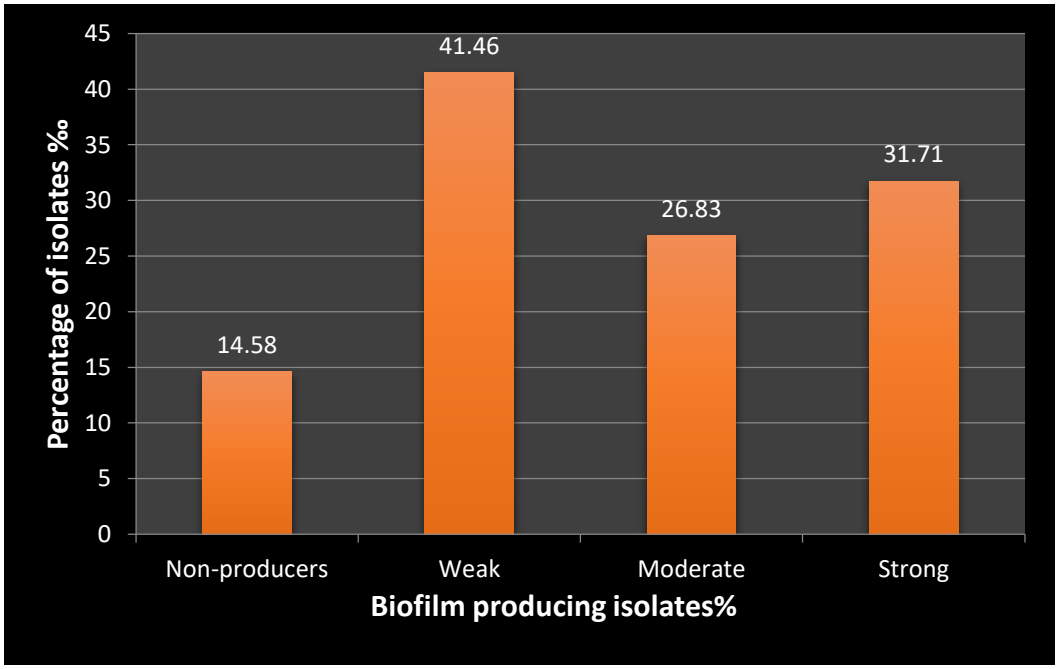

Figure 4. Distribution of environmental *P. aeruginosa* isolates, into non-producers, weak, moderate and strong biofilm producers.

The current study also demonstrated that among the 61 clinical isolates of *P. aeruginosa*, there were strong, intermediate, weak and non-biofilm producing isolates that were isolated from burns, ear infections, urine, and wound (Table 5).

2024, 21(2): 0313-0327
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Baghdad Science Journal

Table 5. Distribution of biofilm-producing clinical *P. aeruginosa* isolates and their sources of isolation.

Isolates source	Isolates	Biofilm formation isolates	Strong (%)	Moderate(%)	Weak (%)	Non producer(%)
Burns	23	21(39.6%)	10(43.5%)	5(38.5 %)	6(40%)	2(25%)
ear infection	20	16(30.2%)	8(34.8 %)	3(23.1 %)	5(33.4%)	4(50%)
urine	11	10(18.9%)	4(17.39 %)	3(23.1 %)	3(20%)	1(12.5%)
wound	7	6(11.3%)	3(17.4 %)	2(25 %)	1(6.6 %)	1(12.5%)
Total no. (61)	61	53(86.9%)	25(47.2%)	13(24.5%)	15(28.3%)	8(13.11%)

Al-Rawi[21] concluded that strong biofilm producing *P. aeruginosa* was found in burn isolates, and the same value was also reported for urinary tract infections, ear infections, and wound infections. Furthermore, Dawood and Ahmed[22] revealed that strong biofilm formation was recorded in burn isolates, then wounds and sputum.

Effects of Temperature on Biofilm Formation

For quantitative estimation of biofilm biomass pre- and post- subjection to changes in temperature, isolates forming biofilms were selected from both clinical and environmental sources. The findings of the current investigation are listed in Table 6. Under the influence of temperature changes, the clinical *P. aeruginosa* isolates showed higher biofilm production as compared to the biofilm of environmental isolates ($P \leq 0.05$).

Table 6. Effect of temperature on the mean biomass of clinical and environmental *P. aeruginosa* isolates (Mean ±SE).

Clinical isolates of *P. aeruginosa*			**Environmental isolates of *P. aeruginosa***			**LSD (P-Value)**
Effect of temperature(OD490)			**Effect of temperature(OD490)**			
37°C	**27°C**	**47°C**	**37°C**	**27°C**	**47°C**	
0.47±0.005	0.45±0.04	0.35±0.01	0.38±0.01	0.44±0.02	0.28±0.006	**0.116 * (0.0392)**
*** (P≤0.05).**						

*significant (P< 0.05)

Obviously, the amount of biofilm formation significantly decreased at high temperatures, possibly as a result of mature biofilms being detached. Given that exopolysaccharides become less viscous as temperature rises, biofilm separation may be caused by the relationship between temperature and viscosity [23]. The results obtained by Donnarumma *et al.*[24] indicated that *Pseudomonas* at 28°C formed weak biofilm, while at 37°C it produced considerable amounts of biofilm. Also, the results obtained by Morimatsu *et al.* [23] found that a significant decrease in the amount of biofilm occurred at high temperatures, while at low temperatures the amount of biofilm was not affected. Other studies revealed that the biofilm formation increased rapidly at temperatures lower than 25°C. *P. aeruginosa* formed the most robust biofilm of a conspicuous mushroom-like structure at 20°C. However, when the temperature increased to 25°C, the biofilm formation rapidly decreased. Above 25°C, as the temperature rose, the biofilm formation increased again little by little despite its less-structured form, indicating that 25°C is the low point of biofilm formation[25].Wu *et al* revealed that biofilm production was found to be maximum at 40°C[26].

Collectively, upon changing the temperature, clinical *P. aeruginosa* isolates showed the highest biofilm production as compared to environmental isolates, and 37°C represented the best temperature for biofilm production.

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Gene expression

P. aeruginosa biofilm genes expression of *pel A*, *psl A*, and *Alg D* were detected as following: various biofilm densities, and different environmental and clinical sources.

Effect of temperature on *pslA* and *pelA* and *AlgD* genes expression on *P. aeruginosa* isolates

The results of the present study summarized in Figs. (5,6,7,8,9) and Tables (7,8,9), revealed that the clinical *P. aeruginosa* isolates had the fold change in the expression level of 0.3 for the *pslA* gene when the temperature was 27°C, while fold change in the expression level increased in environmental isolates to 1.4. When the temperature was 47°C the fold change in expression level of 0.5 but decreased in environmental isolates to 0.18, compared to the control (1.00) when the temperature was 37°C.

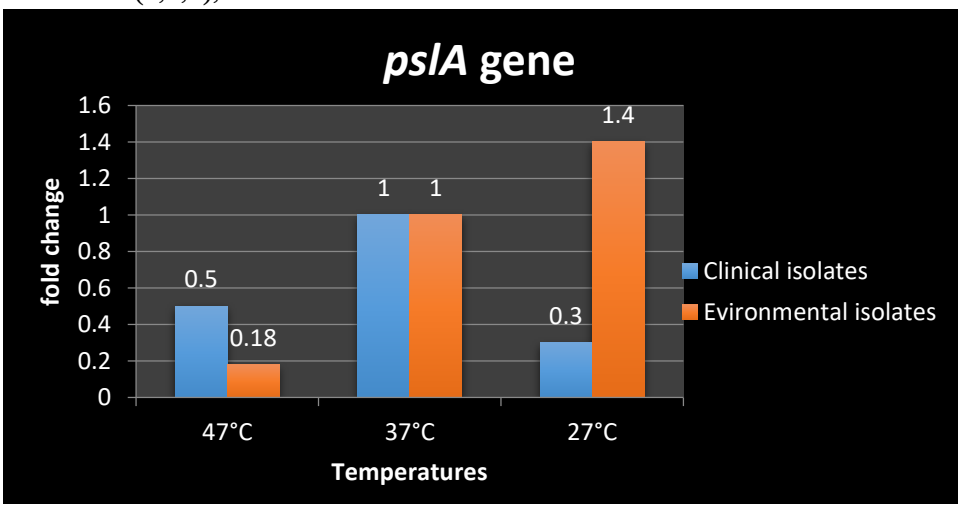

Figure 5. Fold change in the expression of *pslA* gene in clinical and environmental *P. aeruginosa* isolates when the temperature is changed

Table 7. Fold change of *pslA* gene in clinical and environmental *P. aeruginosa* isolates when the temperature is changed.

Isolates	37 °C (Control)			27 °C					° 47C				
	Ct_c PslA	Ct_c 16srRNA	ΔCt_c	Ct_T PslA	Ct_T 16srRNA	ΔCt_T	ΔΔ Ct	Fold change	Ct_T PslA	Ct_T 16srRNA	ΔCt_T	ΔΔ Ct	**Fold change**
Clin.	20.8	9.4	11.4	24.1	11.3	12.8	1.4	0.37	30.2	17.8	12.4	1	**0.5**
Env.	18.6	17.2	1.4	16.3	15.4	0.9	-0.5	1.4	21.6	17.7	3.9	2.5	**0.18**

While in case of *pelA* gene, the fold change in the expression level of clinical isolates (0.18,0.9) when the temperatures 27°C and 47°C, while decreased in environmental isolates to (0.17,0.08) under the same temperatures, compared to the control (1.00) at 37°C.

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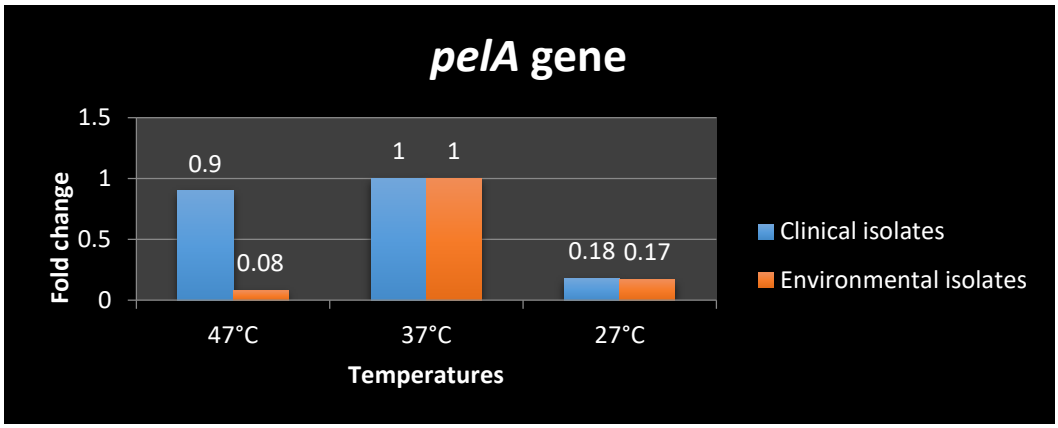

Figure 6. Fold change in the expression of *pelA* gene in clinical and environmental *P. aeruginosa* isolates when the temperature is changed

Table 8. Fold change of *pelA* gene in clinical and environmental *P. aeruginosa* isolates when the temperature is changed.

Isolates	**37 °C (Control)**			**27 °C**					**° 47C**				
	Ctc PelA	Ctc 16srRNA	ΔCtc	CtT PelA	CtT 16srRNA	ΔCtT	ΔΔCt	Fold change	CtT PelA	CtT 16srRNA	ΔCtT	ΔΔCt	**Fold change**
Clin.	15.4	9.4	6	19.7	11.3	8.4	2.4	0.18	23.9	17.8	6.1	0.1	**0.93**
Env.	18.6	17.2	1.4	19.4	15.4	4	2.6	0. 17	22.7	17.7	5	3.6	**0.08**

Also, in case of *algD* gene, the fold change in the expression levels of clinical isolates (0.11,0.5) when the temperatures 27°C and 47°C while decreased in environmental isolates (0.09,0.05) under the same temperatures, compared to the control (1.00) at 37°C.

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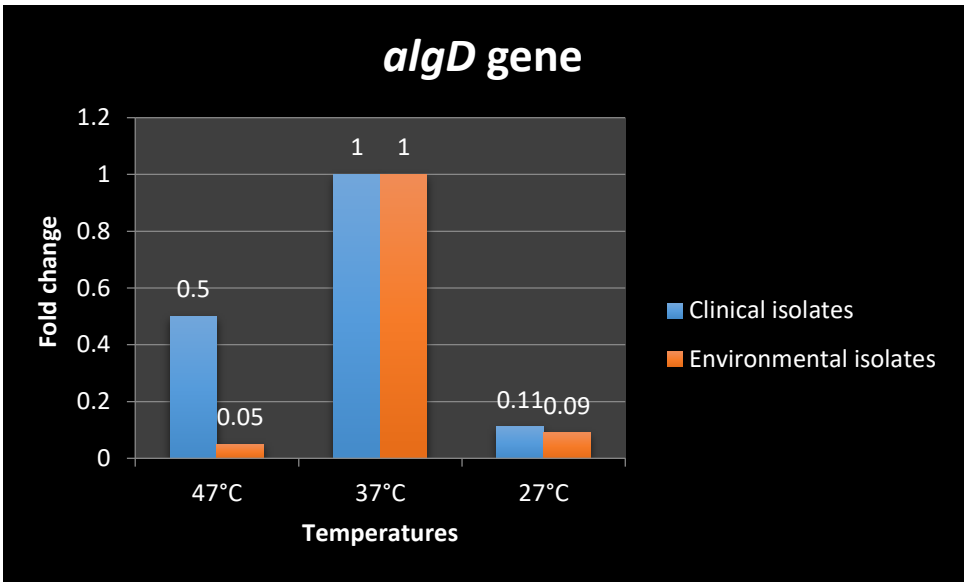

Figure 7. Fold change in the expression of *algD* gene in clinical and environmental *P. aeruginosa* isolates when the temperature is changed

Table 9. Fold change of *algD* gene in clinical and environmental *P. aeruginosa* isolates when the temperature is changed.

Isolates	37 °C (Control)			27 °C					° 47C				
	Ctc algD	Ctc 16srRNA	ΔCtc	CtT algD	CtT 16srRNA	ΔCtT	ΔΔCt	Fold change	CtT algD	CtT 16srRNA	ΔCtT	ΔΔCt	**Fold change**
Clin.	14.6	9.4	5.2	19.6	11.3	8.3	3.1	0.11	24	17.8	6.2	1	**0.5**
Env.	17.7	17.2	0.5	19.4	15.4	4	3.5	0.09	22.4	17.7	4.7	4.2	**0. 05**

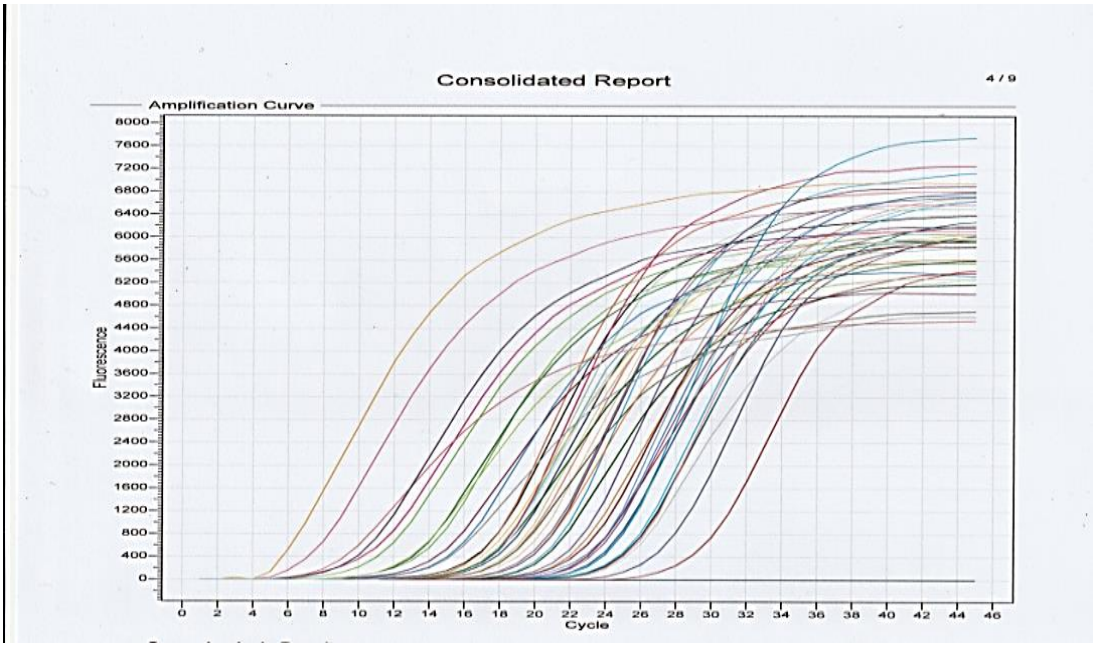

Figure 8. Amplification curve of *pslA, pelA, AlgD,* and *16SrRNA* genes in clinical and environmental *P. aeruginosa* isolates.

2024, 21(2): 0313-0327
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Baghdad Science Journal

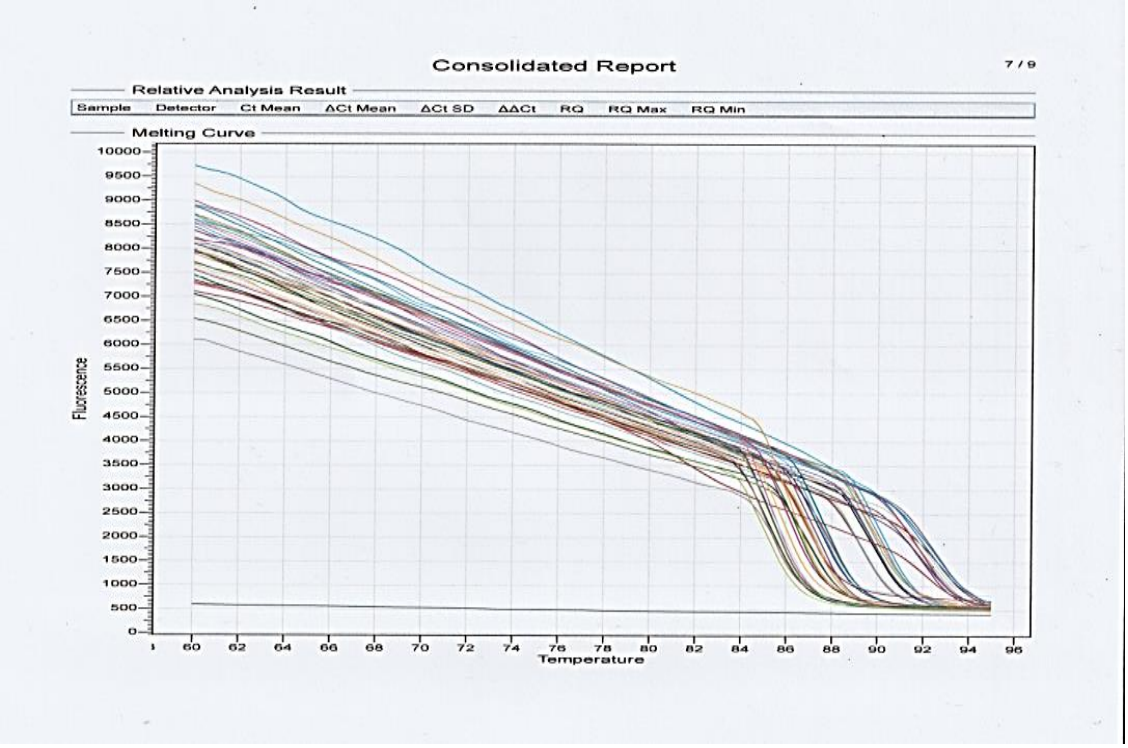

Figure 9. Melting curve of *pslA, pelA, AlgD,* and *16SrRNA* genes in clinical and environmental *P. aeruginosa* isolates.

According to the above results, when the temperature was changed, the gene expression for the environmental isolates decreased rapidly more than for clinical isolates. The reason is that clinical isolates were less affected by temperature change compared to the environmental isolates, and the clinical isolates are more exposed to antibiotics and disinfectants.

The current study revealed that gene expression of *pslA*, *pelA* and *algD* rapidly increased from 27°C to 37°C, reaching a high point at 37°C in the control, and then slightly decreased again at 47°C. The results of this study showed that exopolysaccharides formed at different temperatures, with 37°C producing the most, and 27°C and 47°C producing the least. This finding demonstrates unequivocally that temperature controls the overall exopolysaccharide synthesis. Also, the optimum temperature encourages bacterial growth, hastening the development of biofilm. On the other hand, if the temperature is under the optimal range, bacterial development might be inhibited by reduction response rates, which could thus have an effect on how biofilms form [27]. The present results do not agree with those obtained by Kim *et al*., [25] who found the expression of *Psl*, *Pel* and *alg* genes in *P. aeruginosa* to be greatly increased at 20°C, and the highest levels of exopolysaccharide production were observed at that temperature. Lower levels of exopolysaccharide production were observed at 25, 30, and 37°C. Alva *et al*. compare the expression of some of the biofilm genes, such as *algD*, *pslA*, *pslB*, *pelA*, and *pelD* in environmental and clinical isolates of *P. aeruginosa*, his study revealed the presence of a multidrug-resistant environmental isolate with higher expression of the biofilm genes as compared to the clinical ones[28].In other studies that were conducted to determine the effect of starvation stress on gene expression of *pslA* gene, the results showed the relative change of *pslA* gene expression level (fold change) in clinical and environmental isolates ranging from 3.031 to 4.377 and from 2.085 to 2.969, respectively[29]. Also, Al – Sheikhly *et al*. found the expression of *pelA* and *pslA* in clinical isolates were very low, even though all biofilms were affected by gentamicin, the results of fold change showed a wide variation[30].

2024, 21(2): 0313-0327
https://doi.org/10.21123/bsj.2023.7709
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Baghdad Science Journal

Conclusion

Clinical *P. aeruginosa* isolates indicated the highest biofilm production and genes expression of *pslA*, *pelA* and *algD* compared to the environmental isolates under different temperatures. In addition, both clinical and environmental isolates formed biofilm and carried *pslA*, *pelA* and *algD* genes regardless of the intensity of the biofilm. Furthermore 37°C represented the best temperature for biofilm production.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' Contribution Statement

A.A.H.A. and A.M.R. contributed equally to the design, implementation, analysis of the results, and writing of the manuscript.

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دراسة جزيئية لعلاقة التعبير الجيني لبعض الجينات بتغير درجات الحرارة لنمو البكتيريا

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الخلاصة

تعد الزائفة الزنجارية أحد اهم مسببات الأمراض الانتهازية المسؤولة عن العديد من الاصابات. يتكون الغشاء الحياتي في الزائفة الزنجارية على الاقل من ثلاث انواع من عديد السكريد هي : psl, pel, alginate .جرى فحص تأثير درجة الحرارة على تكوين الغشاء الحياتي والتعبير الجيني باستخدام طريقة فحص الصفيحة الدقيقة وتفاعل البلمرة المتسلسل اللحظي الكمي. من بين 303 عينة سريرية وبيئية شخصت61 عزلة سريرية و48 عزلة بيئية للزائفة الزنجارية. لوحظت قابلية هذه العزلات على تكوين الغشاء الحياتي في 86.9٪ و 85.42٪ من العزلات السريرية والبيئية، على التوالي، تراوحت بين انتاج قوي، ومتوسط، وضعيف للغشاء الحياتي. بالإضافة إلى ذلك، أظهرت العزلات السريرية إنتاجية اعلى للغشاء الحياتي والتعبير الجيني ل *pslA*، *pelA* ، *algD* مقارنةً بالبيئية تحت تأثير تغير درجة الحرارة. تستنتج من هذه الدراسة، بأنه كل من العزلات السريرية والبيئية كونت الغشاء الحياتي وتمتلك الجينات *pslA*، *pel* ، *alg D* بغض النظر عن كثافة هذا الغشاء، وان درجة 37 مئوية تمثل أفضل درجة حرارة لإنتاج الأغشية الحياتية

الكلمات المفتاحية: ، الغشاء الحياتي، التعبير الجيني، *pel A*، *pslA*، الحرارة.