Original Article

Molecular Study of Quercetin-Resistant *Pseudomonas aeruginosa* Isolated from Wound Infections

Khalil Khalis Jafar, Abdul Muhsin M. Shami

Department of Biotechnology, Institute of Genetic Engineering and Biotechnology for the Postgraduate Studies, University of Baghdad, Baghdad, Iraq

Abstract

Background: Quercetin, a crucial plant flavonoid, exhibits a range of pharmacological properties. A significant body of literature explores the antibacterial activity of the substance and its potential mode of action. Quercetin has shown the capacity to inhibit the growth of several types of gram-positive and gram-negative bacteria, as well as fungi and viruses. **Objectives:** The work presented sought to identify siderophore genes in quercetin-resistant *Pseudomonas aeruginosa.* Bacterial isolates from swab samples taken from patients suffering from complicated wound infections. **Materials and Methods:** A total of 200 swab samples were collected from attending patients. Compared to patients who are male (41%), the majority of patients (59%) were female. The patients have provided swab samples for sterile swab transport, displaying signs of a wound infection. The samples were cultivated on nutrient agar, blood agar, MacConkey agar, and cetrimide agar, and they were incubated for 24 h at 37°C. **Results:** The results were recorded by isolating gram-negative bacteria and observing developed colonies after identifying $n = 59$ (29.5%) isolates from 200 clinical samples as *P. aeruginosa*. The *plcN* and *exoS* genes were found in the isolates chosen. In this study, $n = 10$ (100%) isolates tested positive for the *16s rRNA* housekeeping gene, $n = 8$ (80%) of isolates tested positive for the *plcN* gene, and $n = 8$ (80%) isolates tested positive for the *exoS* gene. **Conclusion:** The high prevalence of *plcN* and *exoS* gene detection in quercetin-resistant strains may be the primary reason for the high virulence factor in connection with quercetin resistance.

Keywords: Antibacterial, antimicrobial activity, mechanism of action, *Pseudomonas aeruginosa*, quercetin

Introduction

Quercetin is a significant phytochemical that falls under the category of flavonoids, which are a type of polyphenol. It is extensively found in a variety of fruits, vegetables, and beverages, as well as in flowers, leaves, seeds, and other sources. It is characterized by its ability to prevent cancer, fight against viruses and microbes, protect the nervous system, reduce inflammation, promote heart health, and combat obesity. Quercetin has a molecular formula of $C_{15}H_{10}O_7$, and its molecular structure includes a ketocarbonyl group. Additionally, the oxygen atom attached to the first carbon is capable of forming salts with acids due to its basic nature. The active groups found in quercetin include a dihydroxy group located between the A ring, an o-dihydroxy group at position B, C ring with a C_2, C_3 double bond, and a 4-carbonyl group. The biological actions of quercetin are mostly ascribed to its active phenolic hydroxyl groups and double bonds.[1,2] The global prevalence of multidrug resistance (MDR) in bacteria has developed as a consequence of the persistent administration of antibiotics for the treatment of bacterial diseases. The pharmaceutical industry has faced a significant problem in treating these MDR pathogens.[3] *P. aeruginosa* is a resilient and adaptable bacterium that can thrive in many conditions and has the ability to cause infections in people when given the opportunity. The success of this bacterium can be attributed to its extensive collection of antimicrobial properties.[4] Recently, the scientific community has shown interest in the beneficial effects

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_984_24

Address for correspondence: Dr. Khalil Khalis Jafar, Department of Biotechnology, Institute of Genetic Engineering and Biotechnology for the Postgraduate Studies, University of Baghdad, Baghdad 10071, Iraq.
E-mail: khaleel.elias2300m@ige.uobaghdad.edu.iq

Submission: 24-Oct-2024 **Accepted:** 07-May-2025 **Published:** 23-Jul-2025

How to cite this article: Jafar KK, Shami AMM. Molecular study of quercetin-resistant *Pseudomonas aeruginosa* isolated from wound infections. Med J Babylon 2025;22:S55-60.

of bioactive chemicals derived from many sources such as plants, fruits, vegetables, and drinks.[5] Quercetin has been found to possess a direct bacteriostatic effect by dismantling bacterial structures, modifying cell permeability, and impeding the binding of nucleotides to proteins.[2,6] Monomers and low-molecular-weight compounds obtained from Chinese herbs and plants are considered excellent resources for the creation of new treatments that target *P. aeruginosa* infections.[7,8] The aim of the study was to identify siderophore genes in quercetin-resistant *P. aeruginosa* isolates from swab samples taken from patients suffering from complicated wound infections.

Materials and Methods

Collection of samples

Two hundred wound infection swab samples from patients with infections of all ages and genders were collected from the Al-Kindi Teaching Hospital, Al-Yarmok Teaching Hospital, and Medical City Baghdad, Iraq (Al-Burns Specialist Hospital), from early November 2023 to late February 2024. Identification of *P. aeruginosa* using manual biochemical tests and the VITEK2 compact system.

Isolation and identification of *Pseudomonas aeruginosa*

By using sterile cotton swabs, samples were collected and transported to the laboratory immediately. The swabs were streaked on nutrient, MacConkey, blood, and selective medium *Pseudomonas* cetrimide agar plates and then incubated overnight at 37°C to study the morphology of colonies. The colonies that were suspected of *P. aeruginosa* were processed further for conventional biochemical tests: indole, cytochrome oxidase, catalase, motility, and citrate utilization tests. The VITEK 2 system and molecular markers based on the amplification of *16S rRNA* were used to characterize and confirm the identification of presumptive *P. aeruginosa* isolates.[9]

Minimum inhibitory concentration (MIC) of *Pseudomonas aeruginosa*

Determined by the microtiter plate technique and broth dilution method against quercetin extract. A loopful (1 μL) culture was streaked on Mueller-Hinton broth plates containing a concentration of quercetin at 1024–2 μg/mL; next, the sub-minimum inhibitory concentration (MIC) of quercetin was identified. One milliliter of sterile saline was transferred into a test tube, and a few isolated colonies were transferred from the plates to the saline tube using sterile cotton swabs. The suspension was adjusted to the McFarland standard, desired by the 0.5 McF.[10,11]

Quantitative minimum inhibitory concentration

1. About 100 μL broth was carefully measured out using a micropipette to fill each well in a microtiter plate.
2. The suitable quercetin solutions are applied to the wells in column A, starting from the leftmost side of the plate, using a 100 μL pipette.
3. Set the micropipette to a volume of 100 μL. Transfer the quercetin mix to the wells in the (A) column by aspirating and dispensing the solution 6–8 times, excluding any splashing.
4. Extract a 100 μL volume from column A and transfer it to column B. To column (C) and continue until only column (H) remains. The same set of criteria for dilution can be applied uniformly across the entire series.
5. Remove 100 μL from the (H) column.
6. Using a micropipette set to 100 μL, distribute bacteria into each well.
7. The plates should be incubated at 37°C.
8. Following the incubation period, a solution of dye resazurin (20 μL) was applied to all the wells of the plate. The plate was then incubated for 30 min to observe any alterations in color. The MIC was obtained by visually observing the broth microdilutions. The MIC is defined as the lowest concentration of the solution at which no color change occurs, namely from blue to pink, in the resazurin broth test [Figure 1].[12]

Primers

The preparation of primers according to the previous studies obtained specific primers for the detection of gene expression (GE) listed in Table 1.

Analysis of quantitative real-time polymerase chain reaction (qRT-PCR) assay

The Qubit® first-step RT-qPCR system (Qubit®-USA) was used to amplify messenger ribonucleic acid (mRNA) particles. The amplification reaction was done using the master amplification reaction listed in Table 2, together with the Qubit second-step RT-PCR and the Qubit®-USA listed in Table 3. Experiments were conducted to synthesize various properties of annealing temperature and cDNA.

Delta delta Ct (ΔΔCt) method

The delta delta Ct (ΔΔCt) method is the most straightforward approach for comparing Ct values between a reference gene and a target gene in relative quantification. This method entails selecting a calibrator sample. The calibrator sample might be either the untreated sample, the sample at the optimum temperature of 37°C, or any sample that one wishes to use for comparison with the unknown samples. Initially, the ΔCt is computed for each sample by comparing

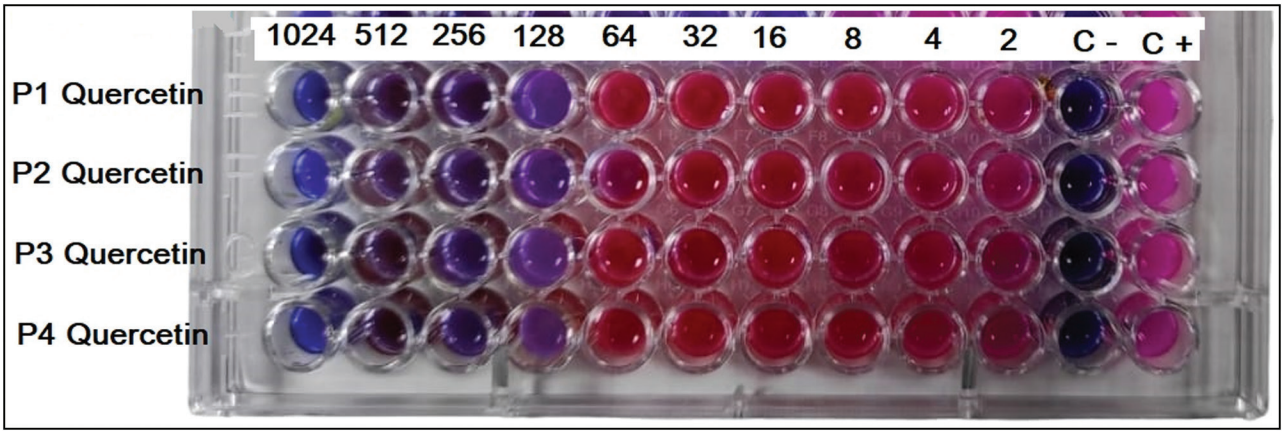

Figure 1: Quantitative estimation of minimum inhibitory concentration quercetin extract for *P. aeruginosa* isolates

Table 1: The primers used in this study

Gene	Oligonucleotide primer sequence (5′ to 3′)	Product size bp.	References
exoS	F: CTTGAAGGGACTCGACAAGG	504	[13]
	R: TTCAGGTCCGCGTAGTGAAT		
plcN	F: GTTATCGCAACCAGCCCTAC	466	[14]
	R: AGGTCGAACACCTGGAACAC		
16s rRNA	F: GGGGGATCTTCGGACCTCA	956	[15]
	R: TCCTTAGAGTGCCCACCCG		

Table 2: Components of reaction polymerase chain reaction (PCR) master mix

PCR master mix reaction components	Volume
GoTaq® Green Master Mix or master mix	12.5 µL
Template DNA	1.5 µL
Primers	
Forward	1 µL
Reverse	1 µL
Nuclease-free (H_2O)	9 µL
Total volume	25 µL

Table 3: Quantitative real-time polymerase chain reaction reaction

Cycle step	Stages	Temperature (°C)	Time
1	Initial denaturation	95	60 s
40–45	Denaturation	95	15 s
	Extension	60	30 s (+ plate read)
1	Melt curve	60–95	40 min

the target gene with the reference gene. This calculation is performed for both the unknown samples and the calibrator sample, as indicated by the following equation.

$$\Delta Ct = Ct \text{ target gene} - Ct \text{ reference gene}$$

The ΔCt of the unknown sample is subtracted from the ΔCt of the calibrator to produce the ΔΔCt value.

$$\Delta\Delta Ct = \text{sample } (Ct \text{ target} - Ct \text{ reference}) - \text{control } (Ct \text{ target} - Ct \text{ reference})$$

The formula $2^{-\Delta\Delta Ct}$ is used to determine the normalized target quantity in the sample. You may use this number to compare the expression levels in different samples. The mRNA expression levels were evaluated by the application of the $2^{-\Delta\Delta Ct}$ comparative cycle threshold (CT) technique. The Livak algorithm was used to gather and evaluate the results.[16]

Ethical approval

This research was successfully carried out in compliance with the moral guidelines found in the Helsinki Protocol. Before taking the specimen, the patient's verbal and written consent was obtained. Document number EC7289, dated 30/11/2023, states that the Research Ethics Committee is in the Genetic Engineering and Biotechnology Institute for Post Graduate Studies. Evaluated and approved the research protocol, subject information, and permission form.

Results

Quantification of *16S rRNA* expression by real-time polymerase chain reaction

The housekeeping gene, *16S rRNA*, whose PCR Ct value was employed in the current investigation before treatment (B.T.), is shown in Table 4. The control group's *16S rRNA*

Table 4: Comparison of *plcN* gene expression between control and quercetin

Sample	CT *16S RNA*	CT *plcN*	ΔCT	ΔΔCT	Fold	Mean
1. B.T.	10.06	14.02	3.96	0	1	1*
2. B.T.	6.57	15.89	9.32	0	1	
3. B.T.	8.27	14.21	5.94	0	1	
4. B.T.	7.82	13.34	5.52	0	1	
P.a.1. A.T. quercetin	5.66	14.21	8.55	4.59	0.04	0.125 ± 0.04
P.a.2. A.T. quercetin	6.42	17.05	10.63	1.31	0.40	
P.a.3. A.T. quercetin	4.44	14.78	10.34	4.4	0.04	
P.a.4. A.T. quercetin	4.81	15.44	10.63	5.11	0.02	

* $P \leq 0.01$

Table 5: Comparison of quercetin and *exoS* gene regulation of gene expression

Sample	CT *16S RNA*	CT *exoS*	ΔCT	ΔΔCT	Fold	Mean
1. B.T.	10.06	37.33	27.27	0	1	1*
2. B.T.	6.57	18.12	11.55	0	1	
3. B.T.	8.27	38.21	29.94	0	1	
4. B.T.	7.82	34.48	26.66	0	1	
P.a.1. A.T. quercetin	5.69	33.23	27.54	0.27	0.82	0.562 ± 0.13
P.a.2. A.T. quercetin	5.78	18.49	12.71	1.16	0.44	
P.a.3. A.T. quercetin	6.42	37.25	30.83	0.89	0.53	
P.a.4. A.T. quercetin	4.81	32.56	27.75	1.09	0.46	

* $P \leq 0.01$

Ct value ranged from 10.06 to 7.82 for the resistant isolates before the use of quercetin.

Quantification of *plcN* expression by real-time polymerase chain reaction

The isolates B.T. in the group had a mean Ct value of 14.02–13.34 for *plcN* B.T. After receiving quercetin treatment (A.T. group), the isolates' *plcN* Ct levels ranged from 14.21 to 15.44. Table 4 indicates that there was a noteworthy variation in the average Ct values among the various study groups. After receiving quercetin therapy, the isolates' mean Ct values were higher than the target gene's mean Ct number on replacing mRNA, its lower expression, and the target gene's lowest copy number carried in mRNA isolates.

Quantification of *exoS* expression by real-time polymerase chain reaction

B.T., the group, the Ct value for *exoS* ranged from 37.33 to 34.48. After treatment with quercetin, the isolates' Ct levels for *exoS* ranged from 33.23 to 32.56. It indicates that the isolates' mRNA samples include the genes. The quercetin group had the highest copy number of the target gene on mRNA, suggesting its altered expression, and the lowest copy number of the target gene carried on mRNA in isolates, as demonstrated by these results. Table 5 shows that there was a significant difference in the average Ct values between the different research groups. The fact that the mean Ct values of the isolates after quercetin treatment differed from those of the isolates before and after treatment indicates that the genes are present in the mRNA samples.

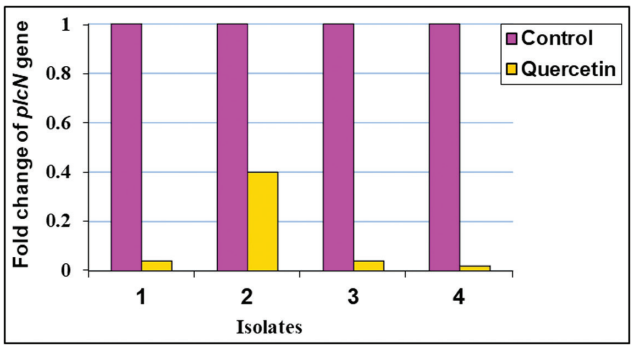

Figure 2: Fold of gene expression of *plcN* gene

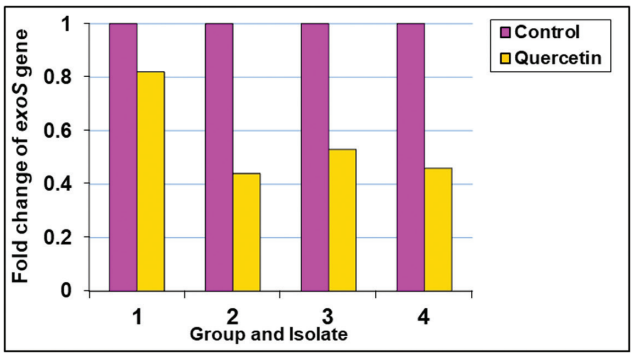

Figure 3: Fold of gene expression of *exoS* gene

Fold gene expression of *plcN* and *exoS*

The results of the study related to gene *plcN* are displayed in Figure 2, and *exoS* gene's fold of expression is depicted in Figure 3. This work used real-time PCR to demonstrate that the gene was expressed by comparing it to isolates that did not receive quercetin treatment and by comparing the results with *P. aeruginosa* isolates that were not given quercetin treatment. The findings corroborated a study that demonstrated the level of expression of a gene; the results indicated a small variation in the fold change of GE.

Discussion

Using real-time PCR quantification, this study used SYBR green fluorescent dye, which intercalates and identifies all double-stranded deoxyribonucleic acid (DNA), including complementary DNA. The amplification was expressed as a CT, or Ct value. Higher Ct values are associated with low GE, while lower Ct values are associated with high GE. The presence of fewer copies is then indicated by the target's higher Ct value, and the reverse is true.[17]

The *16S rRNA* gene was chosen as the housekeeping gene for this experiment because it exhibits continuous expression in the cells under investigation across a range of circumstances.[18] In the current quantitative PCR investigation, four isolates of *P. aeruginosa* resistant to quercetin that share both *plcN* and *exoS* genes were used. In this study, untreated and treated samples of resistant bacteria that were grown using quercetin were compared to analyze the mRNA expression of the *plcN* and *exoS* genes using the qRT-PCR assay.[19,20]

The amplification values of the genes' Ct values were listed from the quantitative RT-PCR. Relative quantification from the ΔΔCt value was used to calculate the fold change in GE in the bacterial samples for the *PlcN* gene and *exoS* gene.[21] At a dose of 64 µg/mL for samples (1, 2, 3, and 4), respectively, quercetin was in the sub-MIC. By using real-time PCR to compare the folds of *plcN* and *exoS* GE with controls that were not treated with quercetin, it was shown that there was an expression level for both genes.[22,23]

Conclusion

This study concluded that most local clinical isolates of *P. aeruginosa* had a resistance percentage to quercetin. This study found that the GE fold was measured by (qPCR) real-time PCR, and the results for both genes (*plcN* and *exoS*) after treatment with quercetin showed different levels of GE.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Osonga FJ, Akgul A, Miller RM, Eshun GB, Yazgan I, Akgul A, *et al*. Antimicrobial activity of a new class of phosphorylated and modified flavonoids. ACS Omega 2019;4:12865-71.
2. Yang D, Wang T, Long M, Li P. Quercetin: Its main pharmacological activity and potential application in clinical medicine. Oxid Med Cell Longevity 2020;2020:1-13.
3. Abdulla AA, Abed TA, Almuttairi AAH. Molecular analysis of the antiseptic resistance genes among multidrug-resistant *Pseudomonas aeruginosa* isolated from burn patients. Med J Babylon 2024;21:456-61.
4. Al-Charrakh AH, Al-Awadi SJ, Mohammed AS. Detection of metallo-β-lactamase producing *Pseudomonas aeruginosa* isolated from public and private hospitals in Baghdad, Iraq. Acta Med Iran 2016;54:107-13.
5. Yamasaki S, Asakura M, Neogi SB, Hinenoya A, Iwaoka E, Aoki S. Inhibition of virulence potential of Vibrio cholerae by natural compounds. Indian J Med Res 2011;133:232-9.
6. Nguyen TL, Bhattacharya D. Antimicrobial activity of quercetin: An approach to its mechanistic principle. Molecules 2022;27:2494-8.
7. Almalki AJ, Ibrahim TS, Taher ES, Mohamed MF, Youns M, Hegazy WA, *et al*. Synthesis, antimicrobial, anti-virulence and anticancer evaluation of new 5 (4 H)-oxazolone-based sulfonamides. Molecules 2022;27:671.
8. Chadha J, Harjai K, Chhibber S. Repurposing phytochemicals as anti-virulent agents to attenuate quorum sensing regulated virulence factors and biofilm formation in *Pseudomonas aeruginosa*. Microb Biotechnol 2022;15:1695-718.
9. Hamad MA, Abdulgafor AB. Bacterial and molecular detection of *Pseudomonas aeruginosa* in feline otitis externa in Baghdad city. J Surv Fish Sci 2023;10:728-36.
10. Al-Bayati SS, Al-Ahmer SD, Shami AM, Al-Azawi AH. Isolation and identification of *Pseudomonas aeruginosa* from clinical samples. Biochem Cell Archiv 2021;21:3931-5.
11. Carter GR. Essentials of veterinary bacteriology and mycology. Essentials Vet Bacteriol Mycol 1982;30:1-6.
12. Al-Sabagh FS, Ghaima KK. Synergistic effect of antimicrobial peptide LL-37 and ciprofloxacin against multidrug resistant *Pseudomonas aeruginosa* isolated from burn infections. Iraqi J Biotechnol 2022;21:32-8.
13. Bogiel T, Depka D, Rzepka M, Kwiecińska-Piróg J, Gospodarek-Komkowska E. Prevalence of the genes associated with biofilm and toxins synthesis amongst the *Pseudomonas aeruginosa* clinical strains. Antibiotics (Basel, Switzerland) 2021;10:241-5.
14. Bogiel T, Prażyńska M, Kwiecińska-Piróg J, Mikucka A, Gospodarek-Komkowska E. Carbapenem-resistant *Pseudomonas aeruginosa* strains-distribution of the essential enzymatic virulence factors genes. Antibiotics (Basel, Switzerland) 2020;10:8-12.
15. Altaai ME, Aziz IH, Marhoon AA. Identification *Pseudomonas aeruginosa* by 16s rRNA gene for differentiation from other pseudomonas species that isolated from patients and environment. Baghdad Sci J 2014;11:1028-34.
16. Talak MA. Evaluation of gene expression of some toll-like receptors genes among Iraqi meningitis. Iraqi J Biotechnol 2023;22:4-12.
17. Ionascu A, Ecovoiu AA, Chifiriuc MC, Ratiu AC. QDATA-an R application implementing a practical framework for analyzing quantitative real-time PCR data. BioRxiv 2023;11:2011-23.
18. Khazaal SS, Fadhil YB, Talha MH, Al-Alak SK. Detection of some virulence genes among clinical *Pseudomonas aeruginosa* isolates in Baghdad. Rev Res Med Microbiol 2024;35: 107-11.
19. Abd_alkader NN, Al-dragh WA. Gene expression of biofilm formation genes (plcN, bap) of *Acinetobacter baumannii* from wounds and burns patients in Baghdad, Iraq. HIV Nurs 2023;23:456-62.

20. Bunyan IA, Al Husseini RFH. Effect of small-interfering RNA (As1974) and HFq-binding proteins on resistance gene and host microRNA (miR-30C) expression in *Pseudomonas aeruginosa*-infected patients from Iraq. Med J Babylon 2023;20:469-76.
21. Ibrahim AH. Link between some virulnce factors genes and antibacterial resistance of *Pseudomonas aeruginosa*. Iraqi J Agric Sci 2022;53:985-93.
22. Tahmasebi H, Dehbashi S, Nasaj M, Arabestani MR. Molecular epidemiology and collaboration of siderophore-based iron acquisition with surface adhesion in hypervirulent *Pseudomonas aeruginosa* isolates from wound infections. Sci Rep 2022;12:7791-9.
23. Al-Jumaily AKT, Turkie AM. Molecular investigation of gene expression of beta-lactamases enzymes gen for *Pseudomonas aeruginosa* bacter. Iraqi J Agric Sci 2018;49:803-8011.