Int.J.Curr.Microbiol.App.Sci (2022) *11*(05): 51-59

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 05 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1105.008

Inhibition of Quorum Sensing Genes Involved in Biofilm Formation and Other Virulence Factors is a Promising Approach in Combating the Pathogenicity of *Pseudomonas aeruginosa*

Fatma Ibrahim Sonbol, Tarek El-Said El-Banna, Aya Mosilhy Ghazy* and Lamiaa A. Al-Madboly

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Tanta University, Tanta, 31611, Egypt

**Corresponding author*

Keywords

biofilm, motility, *Ps. aeruginosa*, pyocyanin, weak acids

Article Info

Received:
05 April 2022
Accepted:
30 April 2022
Available Online:
10 May 2022

A B S T R A C T

Pseudomonas aeruginosa is an opportunistic pathogen causing severe life threatening diseases and continues to be an important cause of nosocomial infections. Virulence mechanisms of *Ps. aeruginosa*, which causes various types of infections are regulated by quorum sensing. Due to emergence spreading of antimicrobial resistance nowadays. Many researches are focused on the use of compounds targeting Qs that are considered to be a new treatment approach for blocking communication between bacteria and reducing virulence, resulted in improving infection control. In this study, some weak acids including lactic, ascorbic, citric and benzoic acids were selected to evaluate their antimicrobial and antivirulence activities at sub-MICs values on our tested isolates. Our results revealed that both lactic and ascorbic acids affected most tested virulence factors. Specifically, lactic acid showed highly significant effect (P-value < 0.001) on virulence factors of *Ps. aeruginosa* than ascorbic acid. The effect of lactic acid on the level of expression of quorum sensing encoding genes (*lasI, lasR, rhII and rhIR)* of two selected *Ps. aeruginosa* isolates was evaluated using RT-PCR. Interestingly, lactic acid was shown to down-regulate expression of genes involved in the control of pseudomonas QS and the decline expression values reached up to 89% in both tested isolates.

Introduction

Pseudomonas aeruginosa is a strong biofilm forming microbe and a major cause of nosocomial infections associated with a significant number of deaths. The major reasons behind its pathogenicity is quorum sensing (QS) and QS- mediated biofilm formation and release of virulence factors like *LasB* elastase, pyocyanin, *LasA* protease, pyoverdin, rhamnolipids, aliginate, etc (Pompilio *et al.*, 2015). Bacterial communication via quorum sensing (QS) plays an important role in control of virulence

factors (Siehnel *et al.,* 2010), in antibiotic resistance (Fuqua *et al.,* 2002) and in biofilm development (Heydorn *et al.,* 2002). Inhibition of quorum sensing is a promising approach to combat the pathogenicity of *Ps. aeruginosa* and eliminate infections (Hentzer *et al.,* 2002; Umesha and Shivakumar, 2013). This issue, together with the consumer demand for novel molecules with antibacterial properties, have encouraged research towards alternatives, such as organic acids (OA) and other nature identical compounds (NIC). These classes of compounds all have a recognized bactericidal effect at different concentrations against several pathogens (Bonetti *et al.,* 2020).

Organic weak acids have been used to treat infections for thousands of years (Stratford and Anslow, 1998). These acids and their salts have been demonstrated to exhibit a large spectrum of action against Gram-positive and Gram-negative bacteria (Jamilah *et al.,* 2008). Various modes of action of weak acids on bacteria have been described (Bjarnsholt *et al.,* 2015).

It was previously reported that weak organic acids could act in their undissociated forms with penetrating microbial cells and inactivating them by lowering their internal pH level or by interfering with metabolic reactions (Theron and Lues, 2010).

In this study, we evaluate antibacterial and antivirulence activities of some weak acids including lactic, ascorbic, citric and benzoic acids at sub-MICs values on tested *Ps. aeruginosa* isolates

Materials and Methods

Ethics statement

All experiments were conducted according to national and international ethical standards. The experimental protocols, including sample collection from Tanta University teaching hospitals, and consent forms, were revised and approved by the Research Ethics Committee, Faculty of Pharmacy, Tanta University, Egypt.

Bacterial isolates

A total of 25 *Ps. aeruginosa* isolates were collected from different departments of Tanta University hospitals during the period from March 2016 to February 2018. The clinical isolates were subjected to standard biochemical tests including citrate utilization, indole, urease, Kilgler's iron agar (KIA) and methyl red tests. In addition, *Pseudomonas aerginosa* (ATCC 27853) obtained from Microbiology department, Faculty of Pharmacy, Tanta University was used as quality control strain.

Chemicals

All tested acids in this study were obtained from obtained from Sigma-Aldrich, USA.

Determination of minimum inhibitory concentration (MIC) of tested acids

Minimum inhibitory concentrations (MICs) of tested weak acids against different bacterial isolates were determined using the agar dilution method recommended by CLSI (2016). Final concentrations that ranged between 0.1 - 12 mg/ml of each weak acid was adjusted in MHA medium and then 3 µl of bacterial suspension containing approximately 10^5 CFU/mlof each organism was spot inoculated with a micropipette and thereafter incubated at 35°C for 24 hours and the MIC was determined. as the lowest concentration of weak acid inhibiting visible growth of each organism on the agar plate Inhibition of bacterial growth in plates containing tested weak acids was judged by comparison with growth in blank control plates Experiments were carried out in triplicate (Thool *et al.,* 2014).

Impact of weak acids on biofilm formation

The tested isolates were screened first for biofilm formation using the crystal violet assay acordint to (Piechota *et al.,* 2018). With some modification. Each assay was performed three times and the results were averaged. Values of absorbance ≥ 0.12 were regarded as bioflm positive, < 0.2 were

considered weak producers, 0.2-0.4 were moderate producers, and > 0.4 were considered strong producers. The effect of tested weak acids on biofilm formation by the strong biofilm producers was investigated using microtitre plate assay. Briefly, overnight cultures of tested isolates were added into 1 mL of fresh LB medium in the presence and the absence of sub-MICs of tested acids. Bacteria were allowed to adhere and grow without agitation for 24 hours at 30 ºC. After incubation, microtitre plate was emptied by removing the media along with free-floating planktonic cells and the wells were gently rinsed twice with sterile water. The surface-attached cells (biofilm) were stained with 200 µL of 0.1% crystal violet solution. After 15 min, crystal violet solution was discarded completely and wells were filled with 200 µL of 33% glacial acetic acid to solubilize CV from the stained cells. The biomass of biofilm was then quantified by measuring the absorbance at OD 570 nm using a microplate reader.

Impact of tested acids on pyocyanin production

Bacterial cultures were added to ten ml of Luria Burtani(LB) supernatants from *Ps. aeruginosa* culture grown in the presence or absence of sub-MICs of weak acids were collected and pyocyanin was extracted by adding 6 ml of chloroform to the 10 ml culture. The chloroform layer was transferred to a clean tube, and 3.2 ml of 1N HCl was added and gently shaken to collect the pyocyanin to the pink aqueous phase (pyocyanin extracted with 1N HCl turns pink). The OD_{520} of the aqueous solution was measured and pyocyanin concentration was determined by multiplying the measurements by 17.07 (Raoof and Latif, 2010).

Impact of tested acids on motility

Overnight untreated and acid treated cultures of*Ps. aeruginosa* isolates were point inoculated onto swarm agar plates containing glucose (1%), bactoagar (0.5%), bactopeptone (0.6%), and yeast extract (0.2%). The plates were incubated at 37°C in an upright position for 24 hours and after incubation the diameter of swarming zone was measured (Krishnan *et al.*, 2012).

Quantitative real-time PCR

Real-time PCR was used to measure the effect of lactic acid on expression of quorum sensing circuit genes *lasIR* and *rhlIR* intwo selected isolates. Expression of the target genes in both treated and untreated isolates was measured. The expression of the target genes was normalized to the expression of reference gene *rpoD*. Calculation of fold increase and fold decrease in gene expression were calculated according to the following equations (Livak and Schmittgen, 2001):

ΔCt = Ct (Target A treated) _ Ct (Ref B treated)

ΔCt = Ct (Target A control) _ Ct (Ref B control)

$\Delta\Delta Ct = \Delta Ct$ (treated)_ ΔCt control

Normalized target gene expression level= $2^{-\Delta\Delta CT}$

Results and Discussion

Different clinical samples were collected from different departments of Tanta University hospitals, including; sputum, urine, stool, blood and wound swabs. All these biological samples were cultured on MacConkey agar. The non-lactose fermenting colonies were selected and subjected to a panel of standard biochemical tests. Twenty five isolates of *Ps. Aeruginosa* were randomly selected for the current study. The MIC values of tested acids determined by agar dilution method. As shown in Table (1), lactic acid showed relatively high antimicrobial activity with MIC values that ranged from 0.4 to 0.8 mg/ml. On the contrary, benzoic acid showed the least antimicrobial activity with MIC values that ranged between 1.6 and 6.2 mg/ml. The MIC range of citric or ascorbic acids was 0.8-3.2 mg/ml against different tested isolates.

Ps. aeruginosa isolates were screened for biofilm production by crystal violet assay. Strong biofilm producers were selected for determining the antibiofilm activity of tested acids at sub-MICs (1/8,

1/4 and 1/2 MIC). Marked reduction in biofilm production by tested isolates after treating with lactic, ascorbic or citric acids was observed. The percent reduction in biofilm production differed with different tested acids and was directly proportional to weak acid concentration as shown in Figure (1). Also, the effect of sub-MICs (1/8, 1/4 and 1/2 MIC) of tested acids on the ability of *Ps. aeruginosa* to produce pyocyanin was investigated. As shown in Figure (2), The results revealed that the concentration of pyocyanin decreased upon increasing the concentrations of lactic or ascorbic acids. Moreover, *Ps. aeruginosa* isolates (n= 9) that showed wide swarming zone diameter were subjected to sub-MICs (1/8, 1/4 and 1/2 MIC) of tested acids to test their effect on swarming motility. Only lactic and ascorbic acids inhibited swarming motility of *Ps. eruginosa* isolates in a dose dependent manner as shown in Figure (3). While, no detectable inhibition was noticed in case of any of other tested weak acids. From the previous data, it was found that there was statistically significant or highly significant differences between lactic and ascorbic acids on different virulence factors of *Ps. aeruginosa* by using t-test as shown in Table (2). Two isolates (Ps 60 and Ps 75) were selected to study the effect of lactic acid on the expression of QS encoding genes. The relative expression of *lasI, lasR, rhlI and rhlR* genes that are responsible for quorum sensing (QS) in *Ps. aeruginosa* was determined. The fold change (percentage reduction) in expression of *lasI, lasR, rhII*and*rhIR* genes of Ps 60 after treatment with lactic acid was 0.16 (84%), 0.13 (87%), 0.39 (61%) and 0.11 (89%), respectively. On the other hand, The fold change in expression of *lasI, lasR, rhII*and*rhIR* genes of Ps75 after treatment with lactic acid was 0.21 (79%), 0.17 (83%), 0.42 (58%) and 0.19 (81%), respectively.

Eradication of *Ps. aeruginosa* has become increasingly difficult due to its remarkable capacity to resist antibiotics. Therefore, the discovery and development of alternative therapeutic strategies that present novel avenues against *Ps. aeruginosa* infections are increasingly demanded and gaining more and more attention (Pang *et al.,* 2019).

A number of studies have proposed that organic acids and phytobiotics as the essential oils are an interesting alternative to antibiotics use in human and veterinary medicine as well as into carcass decontamination strategies, due to their recognized antibacterial activity (de Nova *et al.,* 2019). In this study, antimicrobial activity of some weak acids including lactic, ascorbic, citric and benzoic acids against tested *Ps. aeruginosa* isolates was investigated. Our data comes in agreement with the result of Stanojević-Nikolić *et al.,* (2016) who recorded that MIC value of lactic acid was 1.25 mg/ml against tested *Ps. aeruginosa*.

Also, it wasrecorded that MIC of citric or ascorbic acids was 1.5 mg/ml against tested *Ps. aeruginosa* (Oladapo and Abiodun, 2014). However, in the study of Pundirand Jain, (2011) it was revealed that benzoic acid exerted moderate activity against all tested Gram-positive and Gram-negative bacterial. Biofilm formation is an important aspect of bacterial virulence which is associated with incomplete penetration of antibiotics and development of bacterial resistance (O'Loughlin *et al.,* 2013).

The present study results concerning effect of weak acids on biofilm formation of selected *Ps. aeruginosa* isolates were similar to results obtained by Przek was *et al.,* (2020) who revealed that ascorbic acid at concentrations of 2.5 µg/ml to 25 mg/ml reduces bacterial growth in the biofilm of *S. aureus, Listeria monocytogenes,* and *E. coli*. Another study conducted by Abbas *et al.,* (2019) who reported thatsodium ascorbate at 5–20 mg/ml inhibited biofilm production in *Ps. aeruginosa* isolates.

In addition, Amrutha *et al.,* (2017*)* reported that maximum inhibition of biofilm formation was recorded at 39.13% with lactic acid in *E. coli* and a minimum of 22.53% with citric acid in *Salmonella* spp. In our study, the effect of sub-MICs of tested weak acids on pyocyanin production was also investigated.

Table.1 Incidence of isolates inhibited by different concentrations of tested weak acids.

Tested weak acid	No (%) of *Ps. aeruginosa* isolates inhibited by tested weak acids at conc (mg/ml)							
	0.1	0.2	0.4	0.8	1.6	3.2	6.2	**12**
Lactic acid	-	-	8(32%)	17(68%)	-	-	-	-
Citric acid	-	-	-	23(92%)	2(8%)	-	-	-
Ascorbic acid	-	-	-	5(20%)	13(52%)	7(28%)	-	-
benzoic acid	-	-	-	-	4(16%)	18(72%)	3(12%)	-

Table.2 Effect of lactic and ascorbic acids on virulence factors of *Ps. aeruginosa* isolates.

Isolate code	Virulence factors	% inhibition in presence of 1/2 MIC *		Paired t-test		Interpretation**
		Lactic acid	Ascorbic acid			
		Mean ± SD	Mean ± SD	T	P-value	
Ps 55	Biofilm	58.52 ± 1.13	52.40 ± 1.15	6.576	0.003	S
	Swarming	63.18 ± 0.40	51.66 ± 1.88	10.394	<0.001	HS
	Pyocyaninie	53.49 ± 1.78	45.83 ± 0.91	6.636	0.005	S
Ps 60	Biofilm	61.43 ± 0.84	47.49 ± 0.73	21.625	<0.001	HS
	Swarming	67.16 ± 0.77	55.46 ± 1.14	14.718	<0.001	HS
	Pyocyaninie	52.87 ± 1.96	43.49± 0.56	7.987	<0.001	HS
Ps63	Biofilm	64.99 ± 1.91	58.44 ± 6.60	1.651	0.174	NS
	Swarming	65.20 ± 1.51	55.00 ± 1.00	9.755	<0.001	HS
	Pyocyaninie	50.08 ± 1.48	41.79 ± 1.56	6.681	0.004	S
Ps68	Biofilm	60.39 ± 0.98	52.55 ± 0.72	11.215	<0.001	HS
	Swarming	63.58 ± 1.39	51.73 ± 1.73	9.273	<0.001	HS
	Pyocyaninie	52.36 ± 1.51	46.04 ± 0.83	6.341	0.003	S
Ps 75	Biofilm	63.00 ± 1.06	55.85 ± 1.05	8.322	<0.001	HS
	Swarming	64.42 ± 1.23	52.42 ± 0.76	14.380	<0.001	HS
	Pyocyaninie	48.55 ± 0.96	40.61 ± 0.90	10.501	<0.001	HS

***SD;** standard deviation, ** **HS**; highly significant, **S**; significant, **NS**; non-significant.

Fig.1 Effect of sub-MICs of acids on optical density (OD) values of produced biofilm

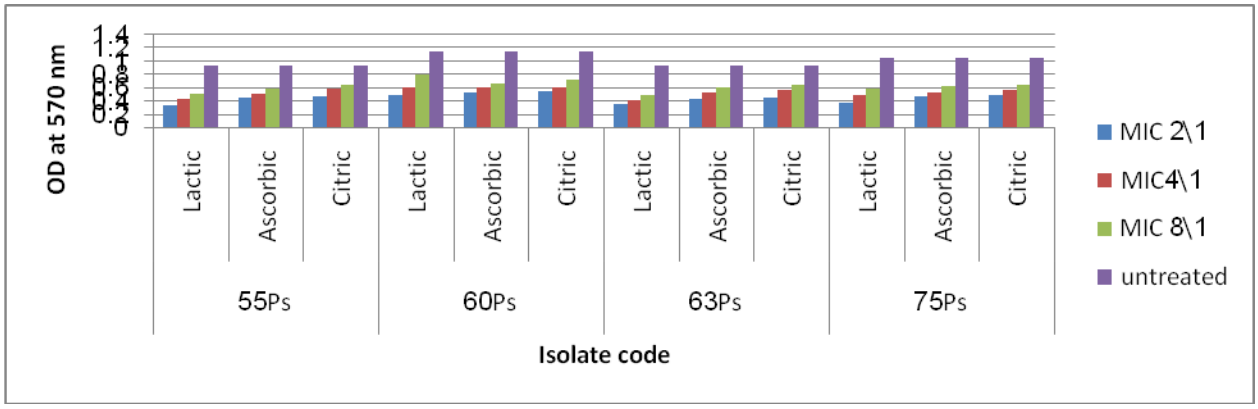


Fig.2 Effect of sub-MICs of tested acids on pyocyanin production

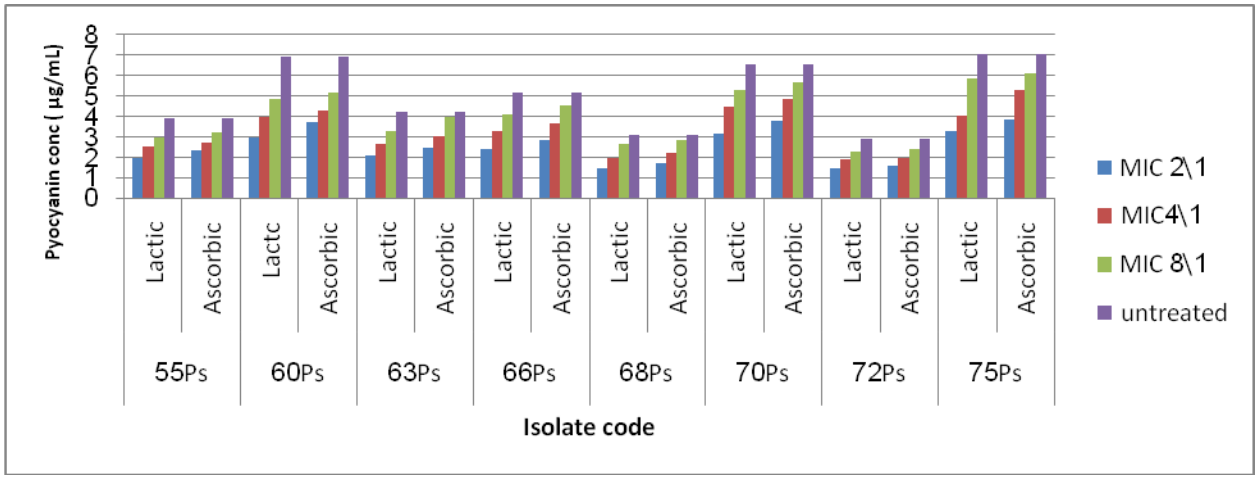
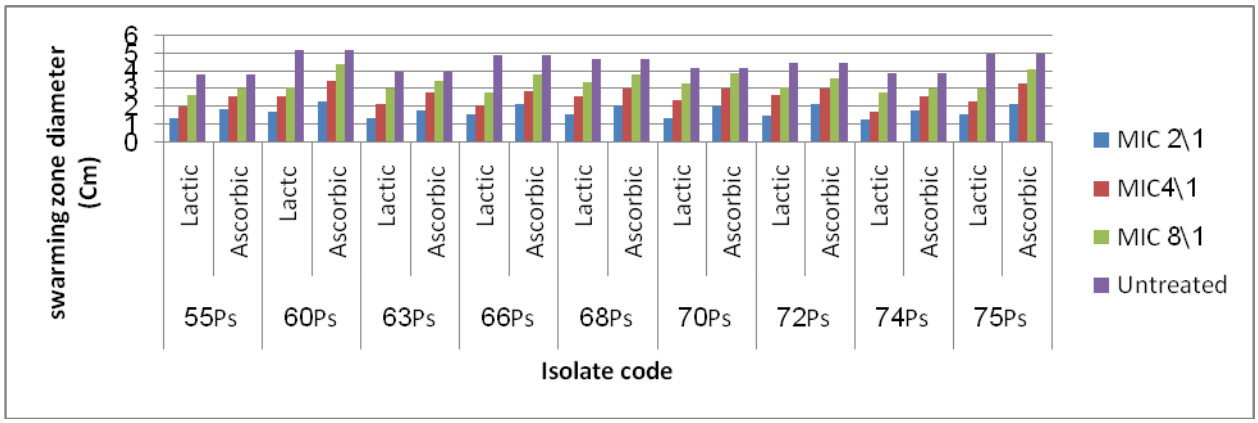


Fig.3 Effect of sub-MICs of tested acids on swarming zone diameter (Cm)



Our data comes in agreement with Kiymaci*et al.,* (2018) who reported that sub-MIC of lactic acid decrease pyocyanin production of *Ps. aeruginoasa*. Our data showed that motility of *Ps. aeruginosa* treated with ascorbic or lactic acids was significantly impaired relative to untreated cell. Similar results was recorded by El-Mowafy *et al.,* (2014)where motility of *Ps. aeruginosa* treated with sodium ascorbate was significantly impaired relative to untreated cell. Also, Burt *et al.,* (2016) reported that 1/2 MIC of lactic acid reduced motility of *Salmonella typhimurium*.The expression of multiple virulence factors which play important roles in the pathogenesis of *Ps. aeruginosa* is regulated by the quorum sensing systems (Gholamrezazadeh *et al.,* 2018).Our findings regarding the significant decrease in biofilm formation, motility and pyocyanin production by *Ps. aeruginosa* isolates by using 1/2 MIC of lactic acid, was explained by evaluation of the effect of lactic acid on the expression of Qs encoding genes (*lasI, lasR, rhII*and *rhIR*) in selected two *Ps. aeruginosa* isolates using Real time-PCR. Our finding agrees with that of Ioana *et al.,* (2013) who revealed that the QS genes expression in *Ps. aeruginosa* multidrug resistant strains decreased in presence of sub-inhibitory concentrations of organic acids. The authors revealed that relative expression level of *lasI, lasR, rhlI, rhlR* genes by RT-qPCR of *Ps. aeruginosa* isolates grown in the presence of sub-inhibitory concentrations of lactic acid was reduced comparatively with those of control strains.

Nowadays, Strategies that minimize unnecessary antibiotic use are needed because so many organisms are becoming more resistant to all types of antibiotics. In this context, we focused on weak organic acids including lactic, citric, ascorbic and benoic acids as they have long been known to have antibacterial activities. Antivirulence activities of tested acids at sub-MIC were also investigated. It was found that citric acid reduced only biofilm production. While, lactic or ascorbic acids inhibited the swarming motility, pyocyanin and biofilm production of *Ps. aeruginosa*. Moreover, it was found tht sub-MIC of lactic acid down regulate the expression of Quorum sensing encoding genes in the selected *Ps. aeruginos*a isolates.

Acknowledgements

My deep thanks and appreciation to my colleagues and technicians and employees in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Tanta University for providing all possible facilities

References

Abbas, H. A., Kadry, A. A., Shaker, G. H., and Goda, R. M. 2019. Impact of specific inhibitors on metallo-β-carbapenemases detected in *Escherichia coli* and *Klebsiella pneumoniae* isolates. *Microbial pathogenesis*. *132*, 266-274.

Amrutha, B., Sundar, K., and Shetty, P. H. 2017. Effect of organic acids on biofilm formation and quorum signaling of pathogens from fresh fruits and vegetables. *Microbial pathogenesis*. *111*, 156-162.

Bjarnsholt, T., Alhede, M., Jensen, P. Ø., Nielsen, A. K., Johansen, H. K., Homøe, P.,... and Kirketerp-Møller, K. 2015. Antibiofilm properties of acetic acid. *Advances in wound care*. *4*(7): 363-372.

Bonetti, A., Tugnoli, B., Rossi, B., Giovagnoni, G., Piva, A., and Grilli, E. 2020. Nature-identical compounds and organic acids reduce *E. coli* K88 growth and virulence gene expression in Vitro. *Toxins*. *12*(8): 468.

Burt, S. A., Adolfse, S. J., Ahad, D. S., Tersteeg-Zijderveld, M. H., Jongerius-Gortemaker, B. G., Post, J. A.,... and Santos, R. R. 2016. Cinnamaldehyde, carvacrol and organic acids affect gene expression of selected oxidative stress and inflammation markers in IPEC-J2 cells exposed to Salmonella typhimurium. *Phytotherapy Research*. *30*(12): 1988-2000.

de Nova, P. J., Carvajal, A., Prieto, M., and Rubio, P. 2019. In vitro susceptibility and evaluation of techniques for understanding the mode of action of a promising non-

antibiotic citrus fruit extract against several pathogens. *Frontiers in Microbiology*. *10*, 884.

El-Mowafy, S. A., Shaaban, M. I., and Abd El Galil, K. H. 2014. Sodium ascorbate as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. *Journal of applied microbiology*. *117*(5): 1388-1399.

Fuqua, C., and Greenberg, E. P. 2002. Listening in on bacteria: acyl-homoserine lactone signalling. *Nature reviews Molecular cell biology*. *3*(9): 685-695.

Gholamrezazadeh, M., Shakibaie, M. R., Monirzadeh, F., Masoumi, S., and Hashemizadeh, Z. 2018. Effect of nano-silver, nano-copper, deconex and benzalkonium chloride on biofilm formation and expression of transcription regulatory quorum sensing gene (rh1R) in drug-resistance *Pseudomonas aeruginosa* burn isolates. *Burns*. *44*(3): 700-708.

Hentzer, M., Riedel, K., Rasmussen, T. B., Heydorn, A., Andersen, J. B., Parsek, M. R.,... and Givskov, M. 2002. Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology*. *148*(1): 87-102.

Heydorn, A., Ersbøll, B., Kato, J., Hentzer, M., Parsek, M. R., Tolker-Nielsen, T.,... and Molin, S. (2002). Statistical analysis of Pseudomonas aeruginosa biofilm development: impact of mutations in genes involved in twitching motility, cell-to-cell signaling, and stationary-phase sigma factor expression. *Applied and Environmental Microbiology*. *68*(4): 2008-2017.

Ioana Cotar, A., Saviuc, C., Andreea Nita, R., Bezirtzoglou, E., Lazar, V., and Carmen Chifiriuc, M. 2013. Anti-pathogenic strategies for fighting *Pseudomonas aeruginosa* infections-probiotic soluble compounds as inhibitors of quorum sensing genes expression. *Current Organic Chemistry*. *17*(2): 155-161.

Jamilah, M. B., Abbas, K. A., and Rahman, R. A. 2008. A review on some organic acids additives as shelf life extenders of fresh beef cuts. *Am. J. Agric. Biol. Sci.* *3*(3), 566.

Kiymaci, M. E., Altanlar, N., Gumustas, M., Ozkan, S. A., and Akin, A. 2018. Quorum sensing signals and related virulence inhibition of *Pseudomonas aeruginosa* by a potential probiotic strain's organic acid. *Microbial pathogenesis*. *121*, 190-197.

Krishnan, T., Yin, W. F., and Chan, K. G. 2012. Inhibition of quorum sensing-controlled virulence factor production in Pseudomonas aeruginosa PAO1 by Ayurveda spice clove (*Syzygium aromaticum*) bud extract. *Sensors*. *12*(4): 4016-4030.

Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*. *25*(4): 402-408.

O'Loughlin, C. T., Miller, L. C., Siryaporn, A., Drescher, K., Semmelhack, M. F., and Bassler, B. L. 2013. A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. *Proceedings of the National Academy of Sciences*. *110*(44): 17981-17986.

Oladapo, A. S., and Abiodun, O. A. 2014. The inhibitory effect of different chemical food preservatives on the growth of selected food borne pathogenic bacteria. *African Journal of Microbiology Research*. *8*(14): 1510-1515.

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., and Cheng, Z. 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnology advances*. *37*(1): 177-192.

Piechota, M., Kot, B., Frankowska-Maciejewska, A., Grużewska, A., and Woźniak-Kosek, A. 2018. Biofilm formation by methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains from hospitalized patients in Poland. *BioMed Research International*, *2018*.

Pompilio, A., Crocetta, V., De Nicola, S., Verginelli, F., Fiscarelli, E., and Di Bonaventura, G. 2015. Cooperative pathogenicity in cystic

fibrosis: *Stenotrophomonas maltophilia* modulates *Pseudomonas aeruginosa* virulence in mixed biofilm. *Frontiers in microbiology*. *6*, 951.

Przekwas, J., Wiktorczyk, N., Budzyńska, A., Wałecka-Zacharska, E., and Gospodarek-Komkowska, E. 2020. Ascorbic acid changes growth of food-borne pathogens in the early stage of biofilm formation. *Microorganisms*. *8*(4), 553.

Pundir, R. K., and Jain, P. (2011). Evaluation of five chemical food preservatives for their antibacterial activity against bacterial isolates from bakery products and mango pickles. *J. Chem. Pharm. Res*. *3*(1): 24-31.

Raoof, W. M., and Latif, I. A. R. 2010. In vitro study of the swarming phenomena and antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from different human infections. *Eur J Sci Res*. *47*(3), 405.

Siehnel, R., Traxler, B., An, D. D., Parsek, M. R., Schaefer, A. L., and Singh, P. K. 2010. A unique regulator controls the activation threshold of quorum-regulated genes in *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences*. *107*(17): 7916-7921.

Stanojević-Nikolić, S., Dimić, G., Mojović, L., Pejin, J., Djukić-Vuković, A., and Kocić-Tanackov, S. (2016). Antimicrobial activity of lactic acid against pathogen and spoilage microorganisms. *Journal of Food Processing and Preservation*. *40*(5): 990-998.

Stratford, M., and Anslow, P. A. 1998. Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid preservative'. *Letters in applied microbiology*. *27*(4): 203-206.

Theron, M. M., and Lues, J. R. 2010. *Organic acids and food preservation*. CRC press. 21-50

Thool, V. U., Wadher, B. J., and Bhoosereddy, G. L. 2014. Citric acid: a prospective permeabilizer for treatment of VISA infections. *International Journal of Current Microbiology and Applied Sciences*. *3*(9): 177-193.

Umesha, S., and Shivakumar, J. 2013. Bcterial quorum sensing and its application in biotechnology. *Int J Pharma Bio Sci*. *4*, 850-861.

How to cite this article:

Fatma Ibrahim Sonbol, Tarek El-Said El-Banna, Aya Mosilhy Ghazy and Lamiaa A. Al-Madboly. 2022. Inhibition of Quorum Sensing Genes Involved in Biofilm Formation and Other Virulence Factors is A Promising Approach in Combating the Pathogenicity of *Pseudomonas aeruginosa*. *Int.J.Curr.Microbiol.App.Sci*. 11(05): 51-59. doi: https://doi.org/10.20546/ijcmas.2022.1105.008