Effect of curcumin and 5-bromoindole-3-carboxyaldehyde on the inhibition of Quorum sensig-dependent virulence factors in *Pseudomonas aeruginosa*

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Abstract

Objetive: To evaluate the inhibitory effect of Quorum Sensing-dependent virulence factors in *Pseudomonas aeruginosa* by 2 molecules; curcumin and 5-bromoindole-3-carboxyaldehyde.

Methods: For the tests, 3 strains of *P. aeruginosa* were used; Pa01, Pa14 and ATCC 27853. The strains were reseeded in LB medium boxes for 12 hours at 37°. ON cultures of each strain were inoculated in LB broth, grown for 24 hours at 37°. Subsequently, new cultures of the strains were inoculated at starting from the ON cultures with an initial O.D 600 nm of approximately 0.0125, the following concentrations of the molecules were evaluated with these cultures: curcumin (1 and 5 µg/ml) and 5-bromoindole-3-carboxyaldehyde (30, 50 and 100 µg/ml). The factors were read virulence with their respective wavelength; protease (595 nm), elastase (495 nm), pyocyanin (520 nm) and alginate (530 nm).

Results: 5-bromoindole-3-carboxyaldehyde significantly inhibited pyocyanin production in Pa01 (92.5% at 100 µg/ml), elastase in Pa01 (79.85% at 100 µg/ml), protease in Pa01 (92.8% at 50 µg/ml), and alginate in ATCC 27853 (76.16% at 100 µg/ml). Curcumin showed substantial inhibition of pyocyanin in ATCC 27853 (100% at 5 µg/ml), elastase in Pa14 (56.19% at 5 µg/ml), protease in Pa01 (87.04% at 5 µg/ml), and alginate in Pa01 (48.26% at 5 µg/ml).

Conclusions: Curcumin and 5-bromoindole-3-carboxyaldehyde demonstrated effective inhibition of QS-dependent virulence factors in *P. aeruginosa*. These findings suggest the potential of these compounds as anti-virulence agents, providing a promising alternative to conventional antibiotics in combating *P. aeruginosa* infections.

Keywords

Quorum sensing, curcumin, antivirulence, bromoindole

Introduction

Pseudomonas aeruginosa is a Gram-negative aerobic rod-shaped bacterium that can be isolated from most environments, including soil, plants, and mammal tissue.[1] Has become an important cause of infection in humans and can be associated with significant morbidity and mortality.[2] Is a common cause of nosocomial infections, particularly pneumonia, infection in immunocompromised hosts, and in those with structural lung disease such as cystic fibrosis.[3,4]

The World Health Organization (WHO) listed *P. aeruginosa* as one of three bacterial species in which there is a critical need for the development of new antibiotics to treat infections.[5] Importantly, *P. aeruginosa* is one of the MDR ESKAPE pathogens.[6]

P. aeruginosa virulence regulation uses both chemical factors such as quorum sensing (QS) and mechanical cues such as surface association to modulate a large suite of virulence factors.[7] Essential function for QS in *P. aeruginosa* is to regulate the production of multiple virulence factors, such as extracellular proteases, iron chelators, efflux pump expression, biofilm development, swarming motility, phospholipases C, alginate, DNase and the response to host immunity.[8,9]

Resistance of *Pseudomonas aeruginosa* to antibiotics is a major problem. Targeting virulence factors is an alternative option to avoid the emergence of resistance to antibiotics and treat infections that this pathogen casuses.[10] QS system an attractive target for antibacterial therapy.[11] Inhibition of this system may cause the attenuation of virulence and protect against infection. In fact, an anti-QS approach has already shown promise in the battle against *P. aeruginosa* infections.[12]

The use of QS inhibitors would be of particular interest in inhibiting bacterial pathogenicity and infections.[13] Turmeric (*Curcuma longa*) has traditionally been used as anti-inflammatory, antimicrobial and anti-fungal, but reports on its anti-QS properties are scarce.[14] Several studies reported that curcumin inhibits bacterial QS systems/biofilm formation and prevents bacterial adhesion to host receptors in various species, including *S. aureus, E. faecalis, E. coli, Streptococcus mutans, Listeria monocytogenes, H. pylori, P. aeruginosa, Serratia marcescens, Aeromonas hydrophila and A. baumannii*.[15]

Another molecule whose reports indicate antibacterial activity are indoles, which, although they use bacteria as signaling, have shown important antibiofilm activity.[16] Functional alteration of indole carboxaldehydes may increase their effectiveness as quorum sensing inhibitors (QSIs). The bromination of carboxyaldehydes represents an increase in their activity.[17]

Given the background of the anti-quorum sensing activity of molecules such as curcumin and carboxyaldehydes, the objective of this work is to evaluate in three strains of *Pseudomonas aeruginosa* (Pa01, Pa14 and ATCC 27853) the activity of curcumin and 5-

bromoindole-3-carboxyaldehyde on quorum-sensing-dependent virulence factors, such as alginate, pyocyanin, elastase and protease.

Methods and materials:

Preparation of the strains and addition of evaluated molecules.

To carry out the tests, 3 different strains of *P. aeruginosa* were used; Pa01, Pa14 and ATCC 27853, all belonging to the microbiology laboratory of the University of Ixtlahuaca CUI. To activate these strains, they were reseeded in boxes of LB medium with 1.5% agar for at least 20 hours at 37°. Subsequently, ON (overnight) cultures of each strain were inoculated in 50 ml flasks with 25 ml of LB medium, cultivated for 18 hours at 37°. After the elapsed time, the growth of the cultures was measured at OD of 600 nm in a 1:10 ratio (dilution in LB).

Subsequently, new cultures of the strains were inoculated from the ON cultures with an OD of 600 nm. The inocula of each strain of interest were added to 3 series of flasks (each with 8 flasks with 25 ml of LB medium) since three different strains were evaluated, different concentrations of each molecule were added to each series: For curcumin (Sigma-Aldrich), they evaluated concentrations of 1 µg/ml and 5 µg/ml, 5-bromoindole-3-carboxyaldehyde (Sigma-Aldrich) 30, 50 and 100 µg/ml. They were incubated at 37° with constant shaking for 24 hours. To maintain control, a culture that did not have any of the added molecules was used. After 24 hours, growth was measured at 600 nm of both the controls and the flasks with the evaluated molecules.

Determination of Pyocyanin.

From the mother flasks with ON, 1000 µl of each culture were taken. They were subsequently centrifuged at 13,000 rpm for 1.5 minutes. 800 µl of the supernatant were taken. Adding 420 µl of chloroform, it was vortexed for 1 minute at speed 10 and centrifuged at 13,000 rpm for 5 minutes. We transferred 300 µl of the bottom phase together with 800 µl of 0.1 N HCl. It was shaken for 1 minute at vortex speed 10, and centrifuged at 13,000 rpm for 5 minutes. It was determined quantitatively by absorbance, measured in a quartz cell, at 520 nm.[18]

Protease Determination.

1000 µl were taken from the stock flasks with ON. The samples were centrifuged at 13000 rpm for 2 minutes. 875 µl of buffer with protease reagent and 125 µl of culture supernatant were used. It was vortexed for 1 minute. Samples were incubated at 37° at 200 rpm for 1 h. At the end of the incubation time, aliquots were taken and placed in 1% $HN0_3$. Shaking with the help of vortex for 1 minute at speed 10, we then centrifuged at 13,000 rpm for 2 minutes. The supernatant was placed in the presence of 0.5% Na0H. We stir lightly with the help of the vortex. Transferring into quartz cells, the samples were read at 595 nm. [19,20]

Determination of alginate.

1000 µl were taken from the stock flasks. 1 ml of the cell culture from the cultures was centrifuged and subsequently heated at 80° for 30 min. The resulting supernatant was centrifuged for 30 min at 13,000 rpm, the pellet was removed, and the alginate was precipitated with ice-cold 99% ethanol. The precipitated alginate was collected and dissolved in 1 ml of 0.9% sterile saline. A mixture of 118 µl of sample plus 1 ml of H_3BO_3/H_2SO_4 and 34 µl of carbazole is made. This mixture was heated for 30 min at 55°C and the absorbance (OD 530) was measured.[21]

Determination of Elastase.

1000 µl were taken from the mother flasks, then centrifuged for 1.5 minutes at 13000 rpm and the supernatant was separated. Subsequently, 100 µl of the supernatant from each of the cultures was placed and 5 mg of elastin-Congo red from Sigma- Aldrich and 900 µl of pH 7.5 buffer were added. They were incubated for 2 h at 37°, centrifuged at 13,500 rpm for 5 minutes, and absorbance was measured at 495 nm. [22]

Statistic analysis.

Two-way analysis of variance (ANOVA) with replication was performed to evaluate the effect of different treatments on bacterial strains Pa01, Pa14, and ATCC27853. The treatments included 5-bromoindole-3-carboxyaldehyde at three different concentrations (30 µg/ml, 50 µg/ml, and 100 µg/ml), and curcumin at two different concentrations (1 µg/ml and 5 µg/ml). Both the ANOVA analysis and the calculation of standard deviation (SD) were performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY).

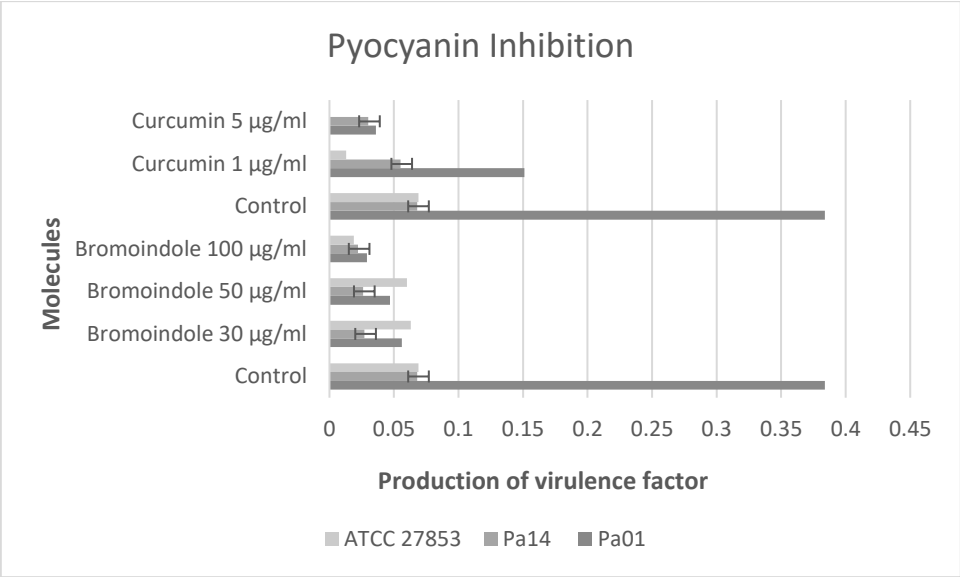
Results

In the present study, the effectiveness of two compounds, 5-Bromoindole-3-carboxyaldehyde and curcumin at different concentrations, in the inhibition of pyocyanin, alginate, elastase and protease quorum-dependent virulence factors in three *Pseudomonas aeruginosa* strains was evaluated: Pa01, Pa14 and ATCC 27853.

Pyocyanin: Bromoindole: Strain Pa01 showed significant inhibition at concentrations of 30 µg/ml (85.42%), 50 µg/ml (87.77%) and 100 µg/ml (92.5%). The Pa14 strain also presented a reduction, although smaller, with values of 60.3%, 61.67% and 67.65% at the same concentrations, respectively. Strain ATCC 27853 showed lower inhibition at 30 µg/ml (8.7%) and 50 µg/ml (13.05%), but higher inhibition at 100 µg/ml (72.47%). Regarding the Pa14 strain at the same concentration. Although the Pa01 strain was the one that produced the highest concentration of pyocyanin in it´s control, it was the strain in which the molecule had the highest activity at all the concentrations studied. On the other hand, the control production of the Pa14 strains and the ATCC 27853 strain varies by 0.001, showing greater activity in the Pa14 strain at concentrations of 30 and 50 µg/ml, but not for the concentration of 100 µg/ml where it is seen. greater inhibition in strain ATCC 27853.

When curcumin was added, strain Pa01 showed an inhibition of 60.68% at 1 µg/ml and 90.63% at 5 µg/ml. The Pa14 strain presented inhibitions of 19.12% at 1 µg/ml and 55.89% at 5 µg/ml. The ATCC 27853 strain showed inhibitions of 81.16% at 1 µg/ml and 100% at 5 µg/ml. Although total inhibition is achieved in strain ATCC 27853, it is the one that showed the lowest pyocyanin production, however, it is the strain with the highest activity, followed by Pa01 and Pa14 respectively. Graph 1.

The two-factor analysis of variance found no significant differences in the inhibition of pyocyanin production between the different treatments with Bromoindole and Curcumin ($F(5, 5) = 1.448$, $p = 0.347$) and between the different bacterial strains evaluated ($F(1, 5) = 0.003$, $p = 0.961$).



Graph 1. Pyocyanin inhibition in the presence of different concentrations of curcumin and 5-bromoindole-3-carboxyaldehyde

Elastase: Bromoindole: For strain Pa01, a significant inhibition was observed with values of 69.77% at 30 µg/ml, 70.64% at 50 µg/ml and 79.85% at 100 µg/ml. The Pa14 strain showed a variable behavior since when adding 30 µg/ml no inhibition was observed, on the contrary it seems that it favored the expression of the virulence factor with 21.11% more compared to the control, an inhibition of 30.42% was shown at 50 µg/ml and 32.99% at 100 µg/ml. The ATCC 27853 strain showed an initial inhibition at 100 µg/ml (31.35%) since, as happened with the Pa14 strain, at concentrations of 30 and 50, greater production of the virulence factor was shown, which suggests that at low concentrations it does not It inhibits but it does stimulate production. Therefore, the molecule showed a high inhibitory effect at all concentrations studied in the Pa01 strain, for the Pa14 strain it is the second in terms of activity and in which it showed the least activity was in the ATCC 27853 strain.

The Pa01 strain presented inhibitions when adding curcumin of 3.6% at 1 µg/ml and 32.08% at 5 µg/ml. Strain Pa14 showed minor inhibitions of 5.93% at 1 µg/ml and 56.19% at 5 µg/ml.

The ATCC 27853 strain presented inhibitions of 5.65% at 1 µg/ml and 18.5% at 5 µg/ml. The molecule at 1 µg/ml presented the least inhibition in the Pa01 strain, and greater activity at both concentrations in the Pa14 strain. Graph 2.

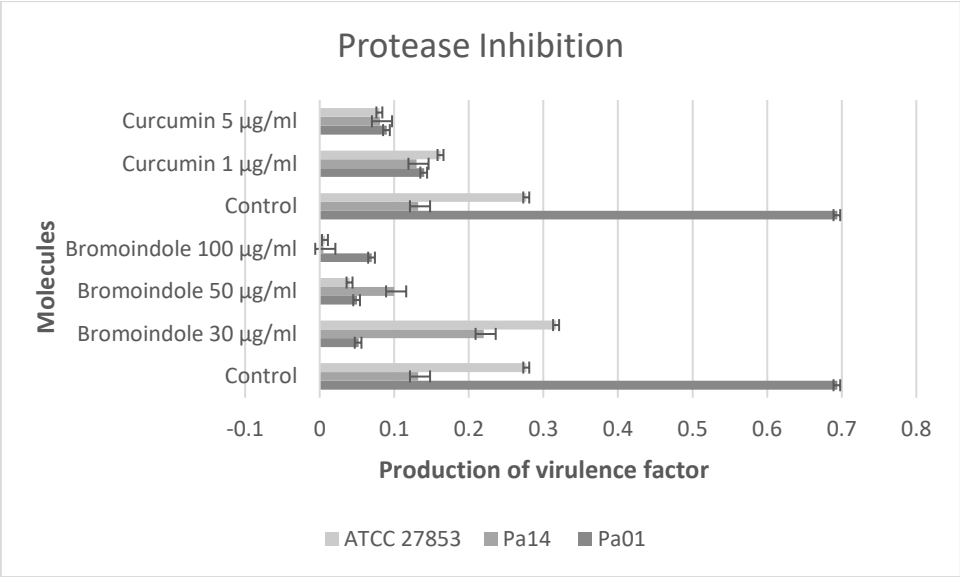
The two-factor analysis of variance did not find significant differences in the inhibition of elastase production between the different treatments with Bromoindole and Curcumin ($F(5, 5) = 3.844$, $p = 0.083$) and between the different strains evaluated ($F(1, 5) = 1.076$, $p = 0.347$). The variability due to error was relatively low (0.0092), indicating that most of the total variability observed in the data can be explained by the molecules, although not significantly in terms of the strains.

Graph 2. Elastase inhibition in the presence of different concentrations of curcumin and 5-bromoindole-3-carboxyaldehyde

Protease: Bromoindole: Inhibition in strain Pa01 was 92.51% at 30 µg/ml, 92.8% at 50 µg/ml and 89.92% at 100 µg/ml. Being the strain where at low concentrations of 30 µg/ml and 50 µg/ml a considerable inhibition is seen, however at 100 µg/m no greater inhibition is seen, on the contrary it seems that it decreases by about 3% compared to concentration No. 2. Strain Pa14 showed variable inhibition, with an initial increase at 30 µg/ml (66.6%), a reduction at 50 µg/ml (24.25%), and almost complete inhibition at 100 µg/ml (96.22%). The ATCC 27853 strain presented an inhibition of 85.56% at 50 µg/ml and 97.48% at 100 µg/ml, although at 30 µg/ml it also showed an increase in the expression of the virulence factor. However, for this virulence factor at concentrations of 30 and 50 µg/ml it is more effective in the Pa01 strain, while at the concentration of 100 µg/ml in the strain that showed the greatest activity in ATCC 27853 and in the one that showed the least activity at the same concentration was Pa01.

For curcumin, the inhibition in strain Pa01 was 79.83% at 1 µg/ml and 87.04% at 5 µg/ml. The Pa14 strain presented inhibitions of 1.52% at 1 µg/ml and 38.64% at 5 µg/ml. The ATCC 27853 strain showed inhibitions of 41.51% at 1 µg/ml and 71.12% at 5 µg/ml. The strain that showed the greatest inhibition of this virulence factor is Pa01, followed by ATCC 27853 and the one that showed the least activity against the molecule was Pa14. Graph 3.

The analysis of variance showed significant differences between the applied treatments in terms of protease activity ($F(5, 5) = 6.786$, $p = 0.028$). This suggests that at least one of the treatments (at different concentrations of Indole and Curcumin) has a significant effect in reducing protease activity compared to the control. The analysis did not indicate significant differences in the response to treatment between the bacterial strains Pa01, Pa14 and ATCC 27853($F (1, 5) = 1.408$, $p = 0.289$). This implies that the strains do not respond differentially to the molecules tested. The variability due to error was relatively low (0.0027), indicating that most of the total variability observed in the data can be explained by the molecules.

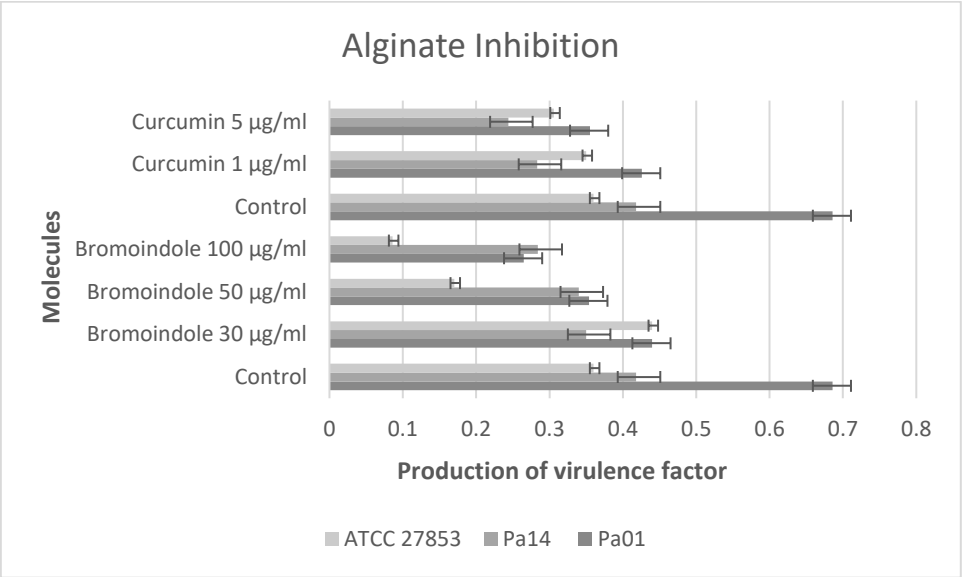


Graph 3. Protease inhibition in the presence of different concentrations of curcumin and 5-bromoindole-3-carboxyaldehyde

Alginate: Bromoindole: Strain Pa01 showed an inhibition of 35.87% at 30 µg/ml, 48.4% at 50 µg/ml and 61.4% at 100 µg/ml. The Pa14 strain presented inhibitions of 16.27% at 30 µg/ml, 18.17% at 50 µg/ml and 40.67% at 100 µg/ml. Strain ATCC 27853 showed an initial increase in alginate production at 30 µg/ml (12.22%), but a significant inhibition at 50 µg/ml (52.78%) and 100 µg/ml (76.16%). The molecule was more active in strain Pa01 and ATCC 27853 at concentrations of 50 and 100 µg/ml, where the greatest activity is seen in the latter strain. For Pa14 it showed the least activity, but with significant inhibition.

With curcumin, strain Pa01 showed inhibitions of 37.91% at 1 µg/mL and 48.26% at 5 µg/ml. The Pa14 strain presented inhibitions of 32.3% at 1 µg/ml and 41.63% at 5 µg/ml. The ATCC 27853 strain showed inhibitions of 2.78% at 1 µg/ml and 15% at 5 µg/ml. Graph 4.

The analysis of variance did not find significant differences in alginate activity between the different treatments with Bromoindole and Curcumin (F (5, 5) = 1.637, p = 0.301). Likewise, no significant differences were observed in the response to treatment between the strains evaluated (F (1, 5) = 0.442, p = 0.536).



Graph 4. Alginate inhibition in the presence of different concentrations of curcumin and 5-bromoindole-3-carboxyaldehyde

Table 1 shows the results in percentage and inhibition concentration of the virulence factors; pyocyanin, elastase, protease and alginate studied with bromoindole. And table 2 shows the results in percentage and inhibition concentration of the virulence factors with curcumin.

Table 1. Inhibition percentages of 5-bromoindole-3-carboxyaldehyde on Quorum-dependent virulence factors

Virulence factors																
Strain/ []	**Pyocyanin**				**Elastase**				**Protease**				**Alginate**			
	Control	30 µg/ml	50 µg/ml	100 µg/ml	Control	30 µg/ml	50 µg/ml	100 µg/ml	Control	30 µg/ml	50 µg/ml	100 µg/ml	Control	30 µg/ml	50 µg/ml	100 µg/ml
Pa01	0 (0.384)	85.42 (0.56)	87.77 (0.047)	92.5 (0.029)	0 (2.282)	69.77 (0.69)	70.64 (0.67)	79.85 (0.46)	0 (0.694)	92.51 (0.052)	92.8 (0.05)	89.92 (0.07)	0 (0.686)	35.87 (0.44)	48.4 (0.354)	61.4 (0.265)
Pa14	0 (0.068)	60.3 (0.027)	61.67 (0.026)	67.65 (0.022)	0 (0.388)	+21.11 (0.47)	30.42 (0.27)	32.99 (0.26)	0 (0.132)	+66.6 (0.22)	24.25 (0.1)	96.22 (0.005)	0 (0.418)	16.27 (0.35)	18.17 (0.34)	40.67 (0.284)
ATCC 27853	0 (0.069)	8.7 (0.063)	13.05 (0.06)	72.47 (0.019)	0 (0.319)	+121.1 (0.708)	+44.22 (0.46)	31.35 (0.219)	0 (0.277)	+14.44 (0.317)	85.56 (0.04)	97.48 (0.007)	0 (0.36)	+12.22 (0.44)	52.78 (0.17)	76.16 (0.086)

Table 2. Inhibition percentages of curcumin on Quorum-dependent virulence factors

Virulence factors												
Strain/ []	**Pyocianin**			**Elastase**			**Protease**			**Alginate**		
	Control	1 µg/ml	5 µg/ml	Control	1 µg/ml	5 µg/ml	Control	1 µg/ml	5 µg/ml	Control	1 µg/ml	5 µg/ml
Pa01	0 (0.384)	60.68 (0.151)	90.63 (0.036)	0 (2.282)	3.6 (2.2)	32.08 (1.55)	0 (0.695)	79.83 (0.14)	87.04 (0.09)	0 (0.686)	37.91 (0.426)	48.26 (0.355)
Pa14	0 (0.068)	19.12 (0.055)	55.89 (0.03)	0 (0.388)	5.93 (0.365)	56.19 (0.17)	0 (0.132)	1.52 (0.13)	38.64 (0.081)	0 (0.418)	32.3 (0.283)	41.63 (0.244)
ATCC 27853	0 (0.069)	81.16 (0.013)	100 (0)	0 (0.319)	5.65 (0.301)	18.5 (0.26)	0 (0.277)	41.51 (0.162)	71.12 (0.08)	0 (0.36)	2.78 (0.35)	15 (0.306)

Discussion

It has been shown that *P. aeruginosa* employs quorum sensing in the regulation of genes encoding extracellular virulence factors. Quorum sensing is an intercellular communication system that allows bacteria to control gene expression in a cell population density-dependent manner.[23]

In *P. aeruginosa*, four fundamental QS pathways have been identified. The first is known as the LasI/LasR cascade. The second includes the RhlI/RhlR pathway. The third is the Pseudomonas quinolone signal (PQS). The fourth is the AI-based integrated quorum sensing system.[24]

In particular, QS blockade abrogates the virulence without affecting bacterial growth. Therefore, QS represents an attractive target for anti-virulence therapy.[25]

In a study conducted by Rudrappa in Pa01 where they evaluated different concentrations of curcumin, at concentrations of 1.5 to 3 µg/ml they achieved an inhibition of pyocyanin of between 60 and 80% of the production with respect to the control, and at 5 µg/ml they achieved an inhibition of 3 times compared to the control. Not only did they achieve an inhibition of pyocyanin, other factors regulated by the QS systems lasI-lasR and rhlI-rhlR were also studied, where they observed a double decrease in the production of elastase and protease, where they also observed that curcumin directly affected the production of the lactones 3-oxoC12-HSL and C4-HSL. In our study we observed that at 1 µg/ml of curcumin we obtained an inhibition for Pa01 of 60% which is similar to that observed for Rudrappa, a value similar to that observed in the ATCC 27853 strain. [26]

Sethupathy and collaborators demonstrated the activity of curcumin at 5 µg/ml on the inhibition of pyocyanin, elastase, protease, alginate and biofilm in 32 clinical isolates and using Pa01 as a control, finding inhibitions that they considered as effective inhibition (50-90%), minimal inhibition (20-49%), no inhibition (0-19%) and induced. Where most of the strains had an effectiveness of between 50 and 90% at said concentration, the factors that showed said inhibition are mainly pyocyanin and elastase. The rest of the strains were maintained with minimal inhibition.[27] In our study at a concentration of 1 µg/ml for Pa01, an inhibition of 60.60% was obtained and for 5 mg of 90.63%, for Pa14 an inhibition of 55.89% was achieved at that concentration, where the greatest activity is seen is in the ATCC 27853 strain where it is achieves 100% pyocyanin inhibition. In addition to similar results in the rest of the virulence factors

A published study reported for Fernandes, that curcumin has inhibitory effects on the LuxS/AI-2 and LasI/LasR QS systems, resulting in a reduction of 33%–77% and 21%, respectively. Significantly, curcumin at high concentration of 200 µg/ml resulted in a 21% decrease in the generation of 3-oxo-C12-HSL by *P. aeruginosa* Pa14 strain.[28]

Liu and collaborators, they monitored pyocyanin production and biofilm formation in *Pseudomonas aeruginosa* when adding curcumin, and they showed a significant decrease in both virulence factors with the aforementioned molecule.[29]

Gholami and collaborators studied the activity of curcumin and curcumin with metal complexes to inhibit quorum and found important decreases in pyocyanin and considerable alginate. Their study shows that curcumin alone reduces pyocyanin production by more than half with a 67.5 % compared to the control in Pa01, and to a lesser extent with metals. On the other hand, the activity in alginate with curcumin alone is lower than that shown in combination with metals with only 21.3%.[30]

Jaduan tested the effect of curcumin mixed with honey to inhibit quorum in *Pseudomonas aeruginosa* in strain Pa01 with the following results: Treatment with curcumin (50 µg/ml) and honey (1%) only showed a weak inhibitory effect on siderophore production, a result similar to that observed in treatment with curcumin alone. Curcumin (50 µg/ml) and honey (1%) alone were not found to significantly reduce the activity of Pa01 metalloproteases elastase y protease. Interestingly, curcumin (50 µg/ml) and honey (1%) alone were ineffective in decreasing alginate production.[31] This synergy shows lower activity than what we obtained in our study for the virulence factors evaluated.

It is known that curcumin acts on the las system. This signaling system is regulated by virulence factors such as the expression of alkaline protease and elastase, which represents the decrease in the production of the autoinducer lactone and therefore the inhibition of production of said factors. As shown in the results.[32] If lasR/lasI system transcriptional regulators expression is affected, other systems such as the PQS and all virulence factors in *P. aeruginosa* will be affected too.[33]

Several studies have described the antibiofilm and antivirulence activities of indole derivatives against clinically important pathogens such as *Pseudomonas aeruginosa*.[34] Halogenation of indoles demonstrates a decrease in IC50 and also greater potency as inhibitors of quorum sensing, as described by Odularu et. al.,[35] The antimicrobial activity of some indolecarboxyaldehydes has been demonstrated in strains of *Pseudomonas sp*, but there is no information on quorum inhibition in *Pseudomonas aeruginosa*.[36]

Among the most studied bacterial species, *C. violaceum* has been shown to affect the production of AHL with good results at concentrations similar to those studied by us.[37]

Indolecarboxyaldehydes such as 5-bromoindole-3-carboxyaldehyde and derivatives in *Vibrio fischeri* have also been shown to inhibit the LuxI/LuxR system, thereby quenching its bioluminescence or biofilm inhibition in *Vibrio parahaemolyticus*.[37,38]

In *Pseudomonas aeruginosa*, the following indole derivatives have been found: Indole, 3-indolylacetonitrile, 7-fluoroindole, indolebased N-acylated L-homoserine lactones and 2-

Aminobenzimidazoles, which can inhibit the production of virulence factors and biofilm formation, however, carboxyaldehyde derivatives with this activity are not reported.[39]

The analyses of variance performed for elastase, protease and alginate activities in Pseudomonas aeruginosa with Curcumin and 5-bromoindole-3-carboxyaldehyde revealed significant differences only in protease activity between treatments. These results suggest that while some compounds may influence the activity of certain enzymes, not all treatments have a uniform impact on all enzyme activities evaluated.

Some limitations of our work are molecular studies to know exactly which gene is repressed and to what extent it is done, however, this represents an opportunity for the future, especially in the case of bromoindole studied, since so far we are the first to report this activity in three different strains of *Pseudomonas aeruginosa*.

Conclusion

This study provides strong evidence that both 5-bromoindole-3-carboxaldehyde and curcumin are effective in inhibiting quorum-sensing-dependent virulence factors in *Pseudomonas aeruginosa* in reference strains such as Pa01, Pa14 and ATCC 27853.

5-bromoindole-3-carboxaldehyde showed significant inhibition of pyocyanin, elastase, protease and alginate production, particularly in strain Pa01, which showed the greatest reductions in the levels of these virulence factors. The inhibition observed in strains Pa14 and ATCC 27853, although notable, was lower, suggesting further studies to elucidate the mechanism.

On the other hand, curcumin is a potent inhibitor of the same virulence factors in all strains studied, with a complete inhibition of pyocyanin production in strain ATCC 27853 at a concentration of 5 µg/ml. However, the efficacy of curcumin is variable between strains, with strain Pa14 showing the lowest overall sensitivity to this compound.

These findings support the hypothesis that inhibition of the quorum sensing system may be an effective strategy to reduce the virulence of *P. aeruginosa* without affecting its growth. The variability observed in the response of the different strains underlines the importance of considering bacterial heterogeneity when developing therapies based on quorum sensing inhibition.

Conflicts of interests

The authors declare that they have no conflict of interest.

Sources of funding

This research work did not receive funding.

Code of ethics

In the present study, reference strains of *Pseudomonas aeruginosa* (Pa01, Pa14 and ATCC 27853) were used to investigate the quorum sensing inhibitory activity of curcumin and 5-bromoindole-3-carboxyaldehyde. All experimental activities were carried out in compliance with BSL-2 level biosafety regulations, strictly following institutional protocols for the safe handling of microorganisms. The use of reference strains in this study does not involve humans or animals, and the samples used are of non-clinical origin and have been widely characterized and documented in the scientific literature. Therefore, ethics committee approval is not required for the performance of this work.

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