ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 03, pp. 34122-34128, March, 2020

RESEARCH ARTICLE OPEN ACCESS

ASSESSMENT OF INDOLE AND PYOCYANIN IN THE RELATIONSHIP OF *PSEUDOMONAS AERUGINOSA* TO *ESCHERICHIA COLI*

Ray Ravilly Alves Arruda, Tarcísio Tarcio Corrêa Bonifácio, Bianca Teixeira Morais de Oliveira, Jonas Emanuel Guimarães da Silva and *Ulrich Vasconcelos

Laboratório de Microbiologia Ambiental, CBIOTEC, Rua Ipê Amarelo, UFPB, Campus I, CEP – 58051-900, João Pessoa-PB, Brazil

ARTICLE INFO

Article History:
Received 08th December, 2019
Received in revised form
19th January, 2020
Accepted 17th February, 2020
Published online 30th March, 2020

Key Words:

Exometabolites, Interspecific Relationships, Amensalism, Biofilms.

***Corresponding author: Ulrich Vasconcelos,**

ABSTRACT

This work evaluated the antiadhesive activity of two microbial exometabolites: indole and pyocyanin. The *in vitro* quantitative microtiter adherence assay was carried out with *Pseudomonas aeruginosa* exposed to concentrations of 0.5 and 1.0 mM of indole and *Escherichia coli* exposed to 0.2 mM of pyocyanin. The incubation took place for 48 hours at 30ºC. Afterwards, the violet crystal test was performed and optical density measurements (590 nm) were used to calculate the percentage of adhesion relative to the control tube, in which the cells were cultured in the absence of exometabolites. Indole and pyocyanindisturbed cell adhesion in all isolates. However, *E. coli* was more sensitive to pyocyanin than *P. aeruginosa* to indole. The percentage of *E. coli* adherence registered an average of around 15%. For *P. aeruginosa*, the percentage of adherence varied from 21 to 54%, with greater activity in the concentration of 1.0 mM of indole. However, adherent cells were detected when the percentage of adherence was close to 20%, suggesting that in an eventual competition between *E. coli* and *P. aeruginosa*, the latter appears to be more tolerant to indole than *E. coli* to pyocyanin.

Citation: ***Ray Ravilly Alves Arruda, Tarcísio Tarcio Corrêa Bonifácio et al.*** **2020.** "Assessment of indole and pyocyanin in the relationship of *pseudomonas aeruginosa* to *escherichia coli*", *International Journal of Development Research*, 10, (03), 34122-34128.

INTRODUCTION

Microbes are dispersed in natural ecosystems and typically live in complex dense communities characterized by distinct patterns of spatial organization, resulting in many benefits (Vasconcelos *et al*., 2020). Nature contains a wide spectrum of possible biotic interactions and microorganisms are no different. Interspecies relationships allow balance to complex heterogeneous communities and are mandatory for the persistence of these organisms in an environment characterized by gradient differences (Zhang, 2019; Godsoe *et al*., 2017). Spatial distribution is an important factor for interspecific bacterial relationships. Natural environments are governed by multiple factors leading to the persistence of dominant groups. In the greater intensity of the disturbance, there is expected to be more stability in the microbial composition (Dohi and Mougi, 2018). In natural environments, bacteria usually compete for space and resources. Thus, ecological interactions of competition and more recently cooperation are recognized as essential factors in regulating the structure and function of microbial communities (Nadell *et al.*, 2016, Hibbing *et al.*, 2010).

Microbial coexistence is widespread in the natural environment, which makes us assume that synergistic interactions between microbial communities are dominant over antagonistic interactions: competition and not cooperation is recognized as predominant (Sun *et al*., 2019). As a result, the competitor's resilience can contribute to increasing population stability (Coyte *et al.*, 2015) even though both inter-specific relationships play important roles in maintaining diversity and population stability (Liu *et al.*, 2016). Antagonistic relationships between *Escherichia coli* and *Pseudomonas aeruginosa* when they share the same environment have been identified. They seem to experience amensalism as the most obvious strategy in the competition for resources (Vasconcelos *et al*., 2010). In terms of ecological typing, amensalism occurs when the growth of *E. coli* is restrained by the coexistence of the two species, while *P. aeruginosa* remains unaffected. Amensalism can still be strong enough to cause the death of *E. coli* (Viana *et al.*, 2017). In their stationary phase, *E. coli* and *P. aeruginosa* can synthesize diffusible exometabolites, such as indole and pyocyanin, who mutually inhibit the exposed cells at a given site (Arruda *et al.*, 2019). Generally, the

molecules included in the natural competition processes for growth inhibition are broad-spectrum antibiotics or bacteriocins (Smid and Lacroix, 2013). The synthesis of these compounds is influenced by the variety of environmental factors, for example limited carbon gradients, as well as by biotic interactions, especially interspecific competition. Despite this, the understanding the physical-chemical and biological properties of these secondary metabolites, as well as the factors that govern their production, is a recent topic of study (Tyc *et al.*, 2017). Indole is one of the most important exometabolites produced by *E. coli* strains from the metabolism of tryptophan (Chu *et al*., 2012). It modulates important physiological characteristics, such as chemotaxis (Bansal *et al.*, 2007), motility (Lopes *et al.*, 2011) and adhesion to surfaces (Hirakawa *et al.*, 2009).

Concentrations between 0.5 to 1 mM can regulate the bacteria's responses to cell stress conditions exerted by coexistence with *P. aeruginosa* (Gaimster *et al.*, 2014). Indole is toxic and can cause membrane derangement. Higher concentrations of indole can even inhibit energy production and protein folding (Kim *et al.*, 2013). Pyocyanin is a brilliant blue-colored phenazine pigment, synthesized exclusively by 90-95% of *P. aeruginosa* strains, acting on iron uptake in pseudomonads (Oliveira *et al.*, 2019), as well as serving as a signaling molecule in response to environmental stress exerted on the bacteria (Dietrich *et al*., 2006). In addition, the exometabolite exhibits antimicrobial activity (Ferguson *et al.*, 2007, Abu *et al*., 2013, Jayseelan *et al.*, 2014), whose mechanism involving oxide-reduction reactions promoting the production and accumulation of peroxide and superoxide ions (O'Malley *et al*., 2003; Bahari *et al*., 2017). The antagonistic relationships between *E. coli* and *P. aeruginosa* that result in disturbances in the formation of biofilms of both species is a relatively well explored theme (Kusnetsova *et al*., 2013; Molina-Santiago *et al*., 2017), however the limitations of the studies occur in how they address the role of indole and pyocyanin in inhibiting the formation of these biofilms. Assuming that both molecules are the most important exometabolites produced by *P. aeruginosa* and *E. coli*, this present study verified the degree of inhibition of cell adhesion from isolates of indole and pyocyanin separately.

MATERIALS AND METHODS

Microorganisms: Five wild isolates were used: two *Pseudomonas aeruginosa* (TGC02 and TGC04) recovered from gas station soils (Cavalcanti *et al.*, 2017) and three *Escherichia coli* (AV02, AV12 and AV14) originated from sink drains in beauty salons (Viana *et al.*, 2017). Two type of cultures, *P. aeruginosa* UFPEDA 416 and *E. coli* UFPEDA 224 were used for comparison purposes. Wild isolates were selected because they meet the criteria of environmental origin with a high degree of selective pressures. These were registered in the National System for the Management of Genetic Heritage and the Associated Traditional Knowledge – SisGen (numbers A6B80BD and ABDD69C).

Exometabolites: High purity indole (Merck KGaA, Darmstadt, Germany) and pyocyanin, produced and extracted from the *P. aeruginosa* TGC04 were used, according to the methodology described by Arruda *et al*. (2019). Briefly, recently cultured cells of TGC04 were suspended in 0.85% NaCl solution (w/v), standardizing the turbidity with tube #1 of the MacFarland scale. Then, 5 mL of the suspension was transferred to flasks containing 50mL of King A broth (King *et al.*, 1954). The flasks were incubated for 72h (150 rpm at 29±1°C). Then, 10 mL was transferred to 3 mL of chloroform. After 1h, 1.5 mL of the chloroform phase was acidified with 1 mL of HCl 0.2 M. After 1h, the acidic phase was neutralized with tris-HCl (pH = 7.2) and the concentration of pyocyanin was estimated by measuring the optical density at λ=520 nm (U2M chemistry), based on a standard curve prepared with 98% pure pyocyanin (Merck KGaA, Darmstadt, Germany) (r = 0.9999).

***In vitro* quantitative microtiter adherence assay:** A crystal violet assay with minor adaptations was used (Khare and Arora, 2011). Briefly, 1.5 µL polystyrene microdilution tubes were filled with 1000 µL of nutrient broth containing the diluted exometabolites and 10 µL of suspension of *P. aeruginosa* or *E. coli* prepared with NaCl 0.85% (w/v) and turbidity standardized with the tube #1 from the MacFarland scale. All tests were performed in triplicate and the controls used nutrient broth without adding exometabolites. Microtubes were statically incubated for 48h at 30°C. After, the broth was discarded and the walls washed 3-5 times with distilled water in order to remove any deposited planktonic cells. The tubes were dried at room temperature for 1h. Afterwards, 1.5 ml of the 1% crystal violet solution (Newprov, Brazil) was added and 20 minutes later, the solution was discarded and the crystal violet excess was removed with vigorous rinsing with distilled water. After the tubes had been dried at room temperature for 1h, 1.5 ml of 95% ethanol was added (Química Moderna, Brazil). Then, 30 minutes later, the optical density of the crystal violet-ethanol solution was measured at 590nm (Shimadzu, UV -1601-1601 PC). Based on the concentrations of indole naturally produced by strains of *E. coli* with activity against *P. aeruginosa*, concentrations of 0.5 and 1.0 mM were tested (Gaimster *et al*., 2014). On the other hand, the activity of pyocyanin against *E. coli* was based on the determination of the minimum inhibitory concentration.

Determination of the Minimum Inhibitory Concentration (MIC) of pyocyanin on *E. coli:* The test was performed using the microdilution technique (Balouri*et al.*, 2016) in order to establish the concentration used in the *in vitro* quantitative microtiter adherence assay with wild and UFPEDA 224 *E. coli*. Briefly, in microdilution plates, a solution of 100 µL pyocyanin was serially diluted in 100 µL of nutrient broth to obtain concentrations from 1.4 to 0.1 mM. Then, 10 µL of the *E. coli* inoculum, prepared in 0.85% NaCl solution, standardized with tube #1 on the MacFarland scale, was added. The plates were incubated at 37°C for 72 hours (with observations every 24 hours). MIC values were determined by visual inspection of turbidity compared to the control (Pffaler *et al.*, 1995). MIC was defined as the lowest concentration at which no bacterial growth was observed. The experiment was carried out in triplicate.

Interpretation criteria: The percent of adhesion was calculated by the difference between the averages of the measurement (triplicate) of the optical densities of the test and the control, divided by the average of the optical density obtained in the control, multiplied by 100. The value found was used to classify the biofilm formation as weak (<40%), moderate (40-80%) or strong (> 80%) (Rodrigues *et al.*, 2010). The interpretative breakpoint value denoting adherent cells was an OD_{590} reading of >0.186. Three times above the mean value of the optical density in the uninoculated medium is

considered to indicate the presence of adherent cells (Pagano *et al*., 2004). In this study the average optical density of the uninoculated medium was 0.062.

Statisticalanalysis: One-way analysis of variance was performed followed by Dunnet's post-test in order to assess the difference in adherence in the presence of exometabolites, compared with the control, considering significant if $p<0.05$. Student t test analysis of variance was used to assess biofilm formation data, considering significant if $p < 0.01$.

RESULTS

Pyocyanin activity on *Escherichia coli* adhesion: A production of 685.0 µg/mL (3.25 mM) of pyocyanin was obtained. All *E. coli* isolates, in contrast, including the strain UFPEDA 224, exhibited sensitivity to the same concentration of the exometabolite, 0.2 mM, at intervals t=24h, t=48h and t=72h. In addition, the inhibitory concentration of pyocyanin significantly interfered with the adhesion of planktonic cells to the polystyrene surface for all *E. coli* tested (Figure 1).

the percentages of adhesion in wild isolates and UFPEDA 224 were similar, obtaining an average of 54.8±6.9%. Comparatively, when correlating this percentage with the results found in the treatments with pyocyanin, the reduction of the adhesion of *E. coli* was significantly different in the inhibitory concentration of the exometabolite.

Indole activity on *Pseudomonas aeruginosa* adhesion: There was a disturbance in the adhesion of *P. aeruginosa* due to the concentration of indole, however, the activity of indole was less harmful to *P. aeruginosa* when compared to the activity of pyocyanin on the adhesion of *E. coli* cells. Exposed to 0.5 mM of indole, the adhesion of wild *P. aeruginosa* isolates was classified as moderate (TGC02=54.4±2.2% and TGC04=43.9±6.8%), to the detriment of the concentration of 1.0 mM (TGC02=25.4±1.5% and TGC04=38.5±2.4%), which led to low adherence, as observed in the strain UFPEDA 416, whose adherence percentages were 21.1±1.2% and 32.3±2.2%, respectively when exposed to 0.5 and 1.0 mM of indole. It is noteworthy that, under all conditions tested, adherent growth was observed (Fig. 2).

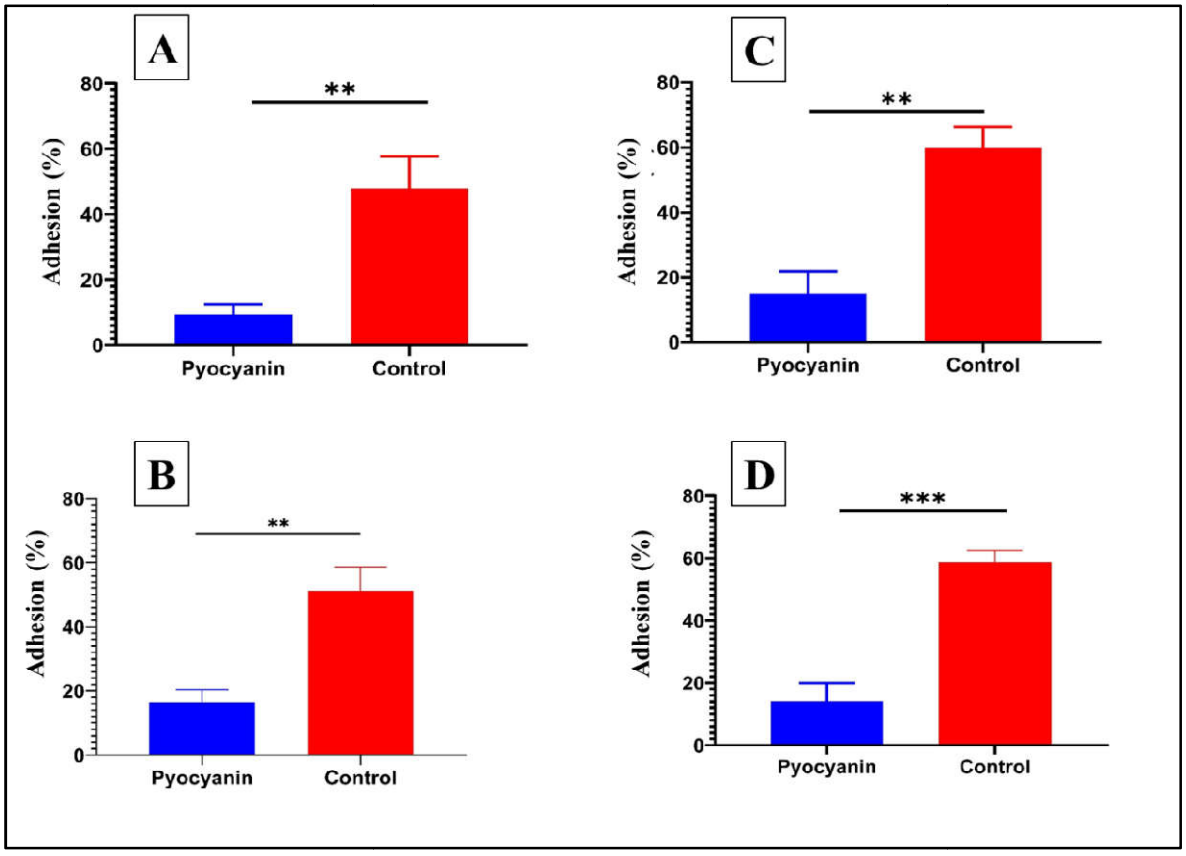

Figure 1. Effect of treatment with the inhibitory concentration of pyocyanin (0.2 mM) on the adhesion of *E. coli* UFPEDA 224 (A), AV12 (B), AV14 (C) and AV02 (D) on the polystyrene surface after 48 hours. Significant differences in control are indicated with an asterisk (p <0.01 and *** p <0.005)**

The adherence percentages of wild isolates AV02 (11.5±5.9%), AV12 (15.6±3.9%) and AV14 (15.3±6.9%), as well as the UFPEDA 224 (10.5±3.1%) were statistically similar. In all cases, adherence was classified as weak. Interestingly, the OD_{590} averages measured in the assays for AV02 (0.172), AV12 (0.128) and UFPEDA 224 (0.137) were below the breakpoint value used to designate adherent cells (OD_{590} reading of >0.186). For the AV14 isolate (0.189), there was a faint presence of adherent cells. In summary, adherence percentages less than about 20% indicated a greater susceptibility of *E. coli* to pyocyanin, inhibiting either cell-surface attachment or cell-cell aggregation. In the test control,

In the absence of indole, the percentages of adherence of wild isolates, relative to control were particularly different between TGC02 (60.2±3.1%) and TGC04 (93.3±2.3%). Comparatively, when correlating the percentage of adherence of the control with the results found in the treatments with indole, the adhesion of TGC02 was significantly different only in the treatment with 1.0 mM. For TGC04, the percentage of adherence was significantly different regardless of the concentration of indole. A similar value was also observed in the control test of the *P. aeruginosa* UFPEDA 416 strain (93.5±2.0%).

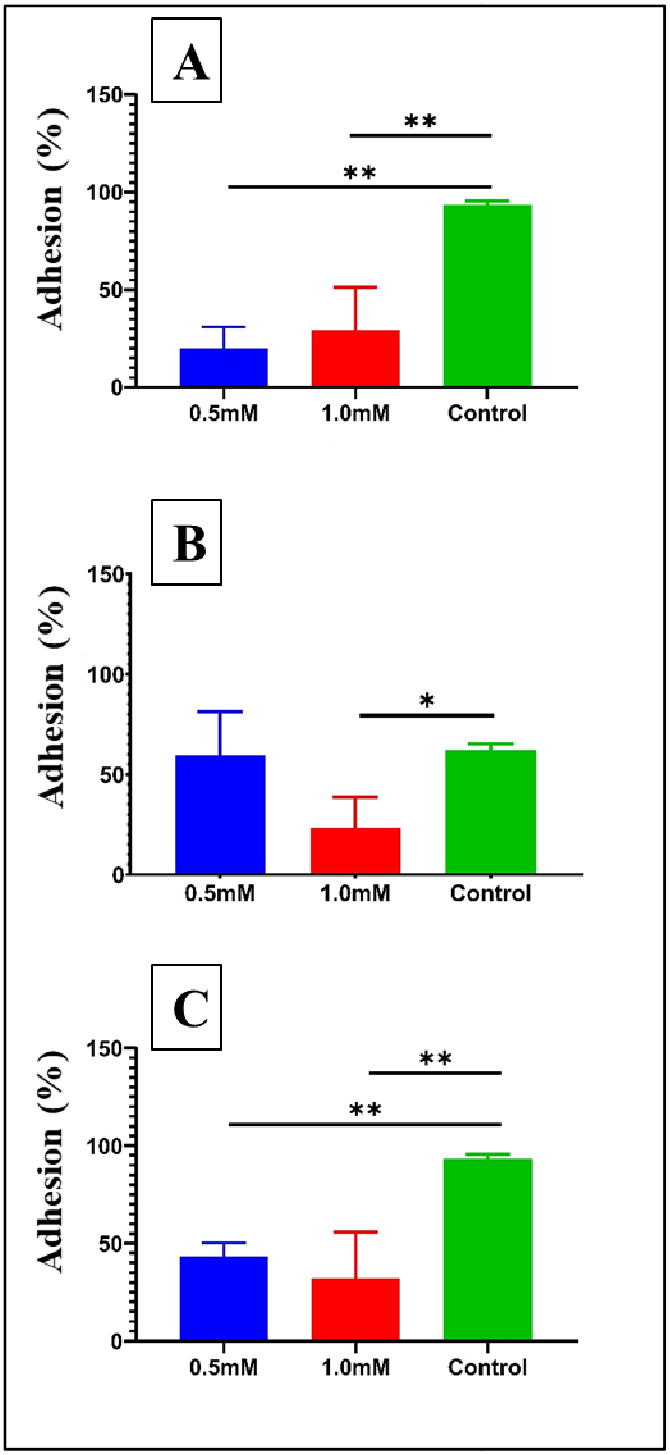

Figure 2. Effect of indole treatment (0.5 and 1.0 mM) on the adhesion of *P. aeruginosa* UFPEDA 416 (A), TGC02 (B) and TGC04 (C) on the polystyrene surface after 48 hours. Significant differences in control are indicated with an asterisk (* p <0.05 and ** p <0.01)

DISCUSSION

Sessile communities are the most prevalent microbial lifestyle in nature. This allows the settlement of mixed communities in coexistence, with a high level of organization, although exhibiting genotypic and phenotypic complexity, distinct from their planktonic forms (Ito *et al.*, 2009; Mittal *et al.*, 2015). This kind of cellular organization offers advantages, such as horizontal changes, protection against moisture loss and proportional increase in the concentration of nutrients. The persistence of the microbial population is guaranteed through different mechanisms, among which the concentration of nutrients and chemical signaling are crucial (Hibbing *et al.*, 2010; Tashiro *et al.*, 2013). *P. aeruginosa* and *E. coli* are two organisms that can coexist in aquatic environments and interaction between them expected (Gonzales-Siles and Sjoling, 2016). The antagonistic relationships between the two species were described in the first classic studies on antibiosis (Hutchison *et al.*, 1943). This association may result in a biostatic effect for the pseudomonads as well as, more dramatically, for enterobacteria (Viana *et al.*, 2017, Martins *et al.*, 2014), although *E. coli* can survive and potentially replicate in nutrient-rich aquatic environments (Ishii and Sadowsky, 2008). Generally, in aquatic environments, there are low levels of essential nutrients for microbial growth, creating a competition site for limited compounds, as well as space (Hirsch, 1986, Mahto and Goel, 2008, Ghoul and Mitri, 2016). This stress scenario will lead a certain microbial group to use its metabolic resources to mitigate selective pressures, becoming dominant and stable through mechanisms driven by competition (Cordero and Datta, 2016). One of the strategies used by microbes is the synthesis of exometabolites with antimicrobial activity. These molecules are generally not toxic to the producing organisms, even in concentrations greater than the physiological concentration seen in stationary-phase (Hassett *et al.*, 1992, Garbe *et al.*, 2000). In addition, subinhibitory concentrations of exometabolites with antimicrobial activity may act as signaling molecules with inter- and interspecies interactions, two of these being indole and pyocyanin (Romero *et al.*, 2011, Jauri *et al.*, 2013).

Pyocyanin is suggested as the most important exometabolite involved in the activity of *P. aeruginosa* against *E. coli* (Angell *et al.*, 2006). Even subinhibitory concentrations of pyocyanin exert important phenotypic changes in enterobacteria, for example in growth kinetics, biochemical profile, mechanisms against oxidative stress, motility and biofilm production (Andrade *et al.*, 2016). Additionally, pyocyanin exhibits good antimicrobial properties against Gram-positive, Gram-negative, fungi and protozoa (Devnath *et al.*, 2017, Kerr *et al.*, 1999). The antimicrobial activity of concentration-dependent pyocyanin has been known for a few decades (Baron and Rowe, 1981). Its action is expected to disrupt the active transport mechanism across the membrane and the respiratory chain of susceptible organisms (Jayaseelan *et al.*, 2014). This results in a decrease in oxygen supply as well as an accumulation of reactive oxygen compounds (Price-Whelan *et al.*, 2007). In addition, pyocyanin synthesized at basal concentrations can mediate complex quorum-dependent mechanisms in *P. aeruginosa* (Mangwani *et al.*, 2015). In this way, the bacteria can regulate the stability of its population when subjected to pressures of different natures, such as exposure to active exometabolites (Bruger and Waters, 2016), which includes adhesion to surfaces and cell aggregation (Skariyachan *et al.*, 2018). Pyocyanin exhibited good activity against adhesion of the *E. coli* to the polystyrene surface. Similar values for the *E. coli* adhesion were also found in a previous study, in which strains of *E. coli* were exposed to linezolid and vancomycin on a polystyrene conditioning surface (Pagano *et al.*, 2004). The establishment of a bacterial biofilm depends on environmental factors that interact with the external bacterial surface as well as intracellular mechanisms (Donlan, 2002). In addition, the time required for the microorganism to generate new cells is crucial for the formation of the biofilm. This represents an important microbial defensive strategy under stressful situations (Chu *et*

al., 2018). In natural environments, some cells that suffer chemical stresses exhibit tolerance phenotypes to certain concentrations of active exometabolites, persisting in the environment, even if there is a slightly reduced growth rate (Heß and Gallert, 2016). It is thus suggested that the UFPEDA 416 strain showed tolerance to 1.0 mM while the *P. aeruginosa* TGC02 exhibited tolerance to 0.5 mM of indole whose percentage of adherence was similar to that observed in the test control, ie, growth condition without addition of the exometabolite. This result was similar to that obtained by a previous study, in which a *P. aeruginosa* strain formed biofilm when showing tolerance to 0.4 mM of indole (Kim *et al*., 2015). This characteristic can be attributed to an anthranilate synthesis by the bacterium. The molecule is involved in the metabolism of tryptophan, and can also participate in the production of pyocyanin, recognized as an important quorum signal molecule of *P. aeruginosa* when exposed to indole (Palmer *et al.*, 2013). On the other hand, indole is the most important signaling molecule in the pyrimidine group synthesized by *E. coli*. The compound is stable and may play a role in the catabolism of amino acids when they become the most important source of energy in nutrient-poor environments (Wang *et al*., 2001). In addition, indole can provide important phenotypic changes in *P. aeruginosa*, as well as for other non-indole-producing microorganisms (Lee *et al*., 2015), including the inhibition of biofilm formation (Frei *et al.*, 2012) and inhibition of antibiotic tolerance (Lee *et al*., 2009). Indole is very toxic to sensitive organisms, possibly leading to changes in membrane permeability as a result of the generation of a superoxide ion (Garbe *et al*., 2000). However, the antimicrobial effect of indole can be suppressed by pseudomonads through the modification or degradation of the molecule by the action of oxygenases (Ma *et al*., 2015). Disregarding the complexity of environments that exert high selective pressures, as well as the interactions between all species that coexist in certain ecosystems, the results suggest that indole and pyocyanin may play an important role in events related to the disturbance of *P. aeruginosa* and *E. coli* biofilm formation. Further investigations need to be conducted to confirm the degree of participation of these exometabolites in the cell aggregation of the two species. It is suggested that the synthesis of indole and pyocyanin represent a clear example that exometabolites produced as metabolic strategies associated with amensalism can contribute to the balance of populations between two competing species since, for both indole and pyocyanin, the cell adhesion to surfaces can be disturbed but not totally inhibited.

Conclusion

Under the conditions evaluated by this study, indole and pyocyanin disturbed the adhesion of *P. aeruginosa* and *E. coli*, however *E. coli* was more sensitive to pyocyanin than *P. aeruginosa* to indole. Additionally, adherent cells were detected under conditions where the percentage of adherence reached values around 20%. Adherences were considered from weak to moderate and the best percentages of adherence were obtained with *P. aeruginosa*, suggesting that in an eventual competition between *E. coli* and *P. aeruginosa*, the second seems to have more advantages and persist for a longer time.

Acknowledgments

The authors would like to thank CAPES and the Graduate Program in Cellular and Molecular Biology for the grants. They also express their gratitude to Dr. GláuciaManoella de Souza Lima, curator for the Culture Collection of the UFPE Antibiotics Department for the supply of UFEDA strains. The English text of this paper has been revised by Sidney Pratt, Canadian, MAT (The Johns Hopkins University), RSAdip - TESL (Cambridge University).

REFERENCES

Abu, E. A., Su, S., Sallans, L., Boissy, R. E., Greatens, A., Heineman, W. R., and Hassett, D. J. 2013. Cyclic voltammetric, fluorescence and biological analysis of purified aeruginosin A, a secreted red pigment of *Pseudomonas aeruginosa* PAO1. *Microbiology.* v. 159, pp. 1736-1747.

Andrade, J. P., Macedo-Farias, L., Ferreira, J. F., Bruna-Romero, O., Glória de Souza, D., Carvalho, M. A., and Santos, K. V. 2016. Sub-inhibitory concentration of Piperacillin-Tazobactam may be related to virulence properties of filamentous *Escherichia coli.* Curr Microbiol.v. 72, pp. 19-28.

Angell, S., Bench, B. J., Williams, H., and Watanabe, C. M. H. 2006. Pyocyanin isolated from a marine microbial population: Synergistic production between two distinct bacterial species and mode of action. *Chem Biol*. v. 13, pp. 1349-1359.

Arruda, R. R. A., Oliveira, B. T. M., Bonifacio, T. T. C., Morais, V. C., Amaral, I. P. G., and Vasconcelos, U. 2019. Activity of two exometabolites produced by *Escherichia coli* on the synthesis of pyocyanin. *Int J Adv Eng Res Sci.* v. 6, pp. 267-271.

Bahari, S., Zeighami, H., Mirshahabi, H., Roudashti, S., and Haghi, F. 2017. Inhibition of *Pseudomonas aeruginosa* quórum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. *J Global Antimicrob Resist.* v. 10, pp. 21-28.

Balouri, M., Sadiki, M., and Ibnsouda, S. K. 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *J Pharm Anal.* v. 6, pp. 71–79.

Bansal, T., Englert, D., Lee, J., Hegde, M., Wood, T. K., and Jayaraman, A. 2007. Differential effects of epinephrine, norepinephrine, and indole on *Escherichia coli* O157:H7 chemotaxis, colonization, and gene expression. Infect Immun. v.75, pp. 4597–4607.

Baron, S. S., and Rowe, J. J. 1981. Antibiotic action of pyocyanin. *Antimicrob Agents Chemother.* v. 20, pp. 814-820.

Bruger, E. L., and Waters, C. M. 2016. Bacterial quorum sensing stabilizes cooperation by optimizing growth strategies. Appl Environ Microbiol. v. 82, pp. 6498-6506.

Cavalcanti, T. G., Souza, A. F., Ferreira, G. F., Dias, D. S. B., Severino, L. S., Morais, J. P. S., Sousa, K. A., and Vasconcelos, U. 2017. Use of agro-industrial waste in the removal of phenanthrene and pyrene by microbial consortia in soil. *Waste Biomass Valor*. https://doi:10.1007/s12649-017-0041-8.

Chu, E. K., Kilic, O., Cho, H., Groisman, A., and Levchenko, A. 2018. Self-induced mechanical stress can trigger biofilm formation in uropathogenic*Escherichia coli.* Nature Comm. v. 9, https://doi:10.1038/s41467-018-06552-z.

Chu, W., Zere, T. R., Weber, M. M., Wood, T. K., Whiteley, M., Hidalgo-Romano, B., Valenzuela, E. J., and McLean, R. J. C. 2012. Indole production promotes *Escherichia coli* mixed-culture growth with *Pseudomonas aeruginosa* by

inhibiting quorum signaling. *J Appl Environ Microbiol.* v. 78, pp. 411–419.

Cordero, O. X., and Data, M. S. 2016. Microbial interactions and community assembly at microscales. *CurrOpin Microbiol.* v. 31, pp. 227-234.

Coyte, K. Z., Schluter, J., and Foster, K. R. 2015. The ecology of microbiome: Networks, competition, and stability. Science. v. 350, pp. 663-666.

Devnath, P., Uddin, M. K., Ahmed, F., Hossain, M. T., and Manchur, M. A. 2017. Extraction, purification and characterization of pyocyanin produced by *Pseudomonas aeruginosa*. *Int Res J Biol Sci.* v. 6, pp. 1-9.

Dietrich, L. E. P., Prince-Whelan, A., Petersen, A., Whiteley, M., and Newman, D. K. 2006. The phenazine pyocyanin is a terminal signaling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Molec Microbiol.* v. 61, pp. 1308-1321.

Dohi, M., and Mougi, A. 2018. A coexistence theory in microbial communities. Roy Soc Open Sci. v. 5, https://doi:10.1098/rsos.180476.

Donlan, R. M. 2002. Biofilms: Microbial life on surfaces. *Emerg Infect Dis.* v. 8, pp. 881-890.

Ferguson, D., Cahill, O. J., and Quilty, B. 2007. Phenotypic, molecular and antibiotic resistance profiling of nosocomial *Pseudomonas aeruginosa* strain isolated from two Irish hospitals. *J Med Biolol Sci.* v. 1, pp. 1-15.

Frei, R., Breitbach, A. S., and Blackwell, H. E. 2012. 2-Aminobenzimidazole derivatives strongly inhibit and disperse *Pseudomonas aeruginosa* biofilms. *Angew Chem Int Ed Engl.* v. 51, pp. 5226-5229. https://10.1002/ anie.201109258.

Gaimster, H., Cama, J., Hernández-Ainsa, S., Keyser, U. F., and Summers, D. K. 2014. The indole pulse: A new perspective on indole signaling in *Escherichia coli*. PLoS One. v. 9, pp. e93168. https://10.1371.

Garbe, T. R., Kobayashi, M., and Yukawa, H. 2000. Indole-inducible proteinsin bacteria suggest membrane and oxidant toxicity. *Arch Microbiol*. v. 173, pp. 78-82. https:// 10.1007/s002030050012.

Ghoul, M., and Mitri, S. 2016. The ecology and evolution of microbial competition. *Trends Microbiol.* v. 24, pp. 833-845.

Godsoe, W., Holland, N. J., Cosner, C., Kendall, B. E., Brett, A., Jankowski, J., and Holt, R. D. 2017. Interspecific interactions and range limits: contrasts among interaction types. *Theor Ecol*. v. 10, pp. 167-179.

Gonzales-Siles, L., and Sjoling, A. 2016. The different ecological niches of enterotoxigenic *Escherichia coli*. Environ Microbiol. v. 18, pp. 741-751.

Hassett, D. J., Charniga, L., Bean, K., Ohman, D. E., and Cohen, M. S. 1992. Response of *Pseudomonas aeruginosa* to pyocyanin: mechanisms of resistance, antioxidant defenses, and demonstration of a manganese-cofactored superoxide dismutase. Infect Immun. v. 60, pp. 328-336.

Heß, S., and Gallert, C. 2016. Growth behavior of *E. coli*, *Enterococcus* and *Staphylococcus* species in the presence and absence of sub-inhibitory antibiotic concentrations: Consequences for interpretation of culture-based data. *Microb Ecol.* v. 72, pp. 898-908.

Hibbing, M. E., Fuqua, C., Parksen, M. R., and Peterson, S. B. 2009. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol.* v. 8, pp. 15-25.

Hirakawa, H., Kodama, T., Takumi-Kobayashi, A., Honda, T., and Yamaguchi, A. 2009. Secreted indole serves as a signal for expression of type III secretion system translocators in enterohaemorrhagic *Escherichia coli* O157: H7. Microbiology. v.155, pp. 541–550.

Hirsch, P. 1986. Microbial life at extremely low nutrient levels. Adv Space Res. v. 6, pp. 287-298.

Hutchison, D., Weaver, R. H., and Scherago, M. 1943. The incidence and significance of microorganisms antagonistic to *Escherichia coli*. *J Bacteriol.* v. 45, p. 29.

Ishii, S., and Sadowsky, M. J. 2008. *Escherichia coli* in the evironment: Implications for water quality and human health. Microbes Environ. v. 23, pp. 101–108. https://10.1264/jsme2.23.101.

Ito, A., Taniuchi, A., May, T., Kawate, K., and Okabe, S. 2009. Increased antibiotic resistance of *Escherichia coli* in mature biofilms. *Appl Environ Microbiol*. v. 75, pp. 4093-4100.

Jauri, P. V., Bakker, M. G., Salomon, C. E., and Kinkel, L. L. 2013. Subinhibitory antibiotic concentrations mediate nutrient use and competition among soil *Streptomyces*. PlosOne. v. 8, p. e81064. https://10.1371/journal.pone.0081064.

Jayaseelan, S., Ramaswamy, D., and Dharmaraj, S. (2014) Pyocyanin: production, applications, challenges and new insights. *World J Microbiol Biotechnol*. v.30, pp. 1159-1168.

Kerr, J. R., Taylor, G. W., Rutman, A., Høiby, N., Cole, P. J., and Wilson, R. 1999. *Pseudomonas aeruginosa* pyocyanin and 1-hydroxyphenazine inhibit fungal growth. J Clin Pathol. v. 52, pp. 385-387.

Khare, E., and Arora, N. K. 2011. Dual activity of pyocyanin from *Pseudomonas aeruginosa* -antibiotic against phytopathogen and signal molecule for biofilm development by rhizobia. *Can J Microbiol.* v. 57, pp. 708–713.

Kim, J., Hong, H., Heo, A., and Park, W. 2013. Indole toxicity involves the inhibition of adenosine triphosphate production and protein folding in *Pseudomonas putida*. FEMS Microbiol Lett. v. 343, pp. 89–99.

Kim, S. K., Park, H. Y., and Lee, J. H. (2015) Anthranilate deteriorates the structure of *Pseudomonas aeruginosa* biofilms and antagonizes the biofilm-enhancing indole effect. Appl Environ Microbiol. v. 81, p. 2328-2338. https://10.1128/AEM.03551-14.

King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med. v. 44, pp. 301-307.

Kusnetsova, M. V., Maslennikova, I. L., Karpunina, T. I., Nesterova, L. Y., and Demakov, V. A. 2013. Interactions of *Pseudomonas aeruginosa* in predominant biofilm or planktonic forms of existence in mixed culture with *Escherichia coli in vitro. Can. J. Microbiol*. v. 59, pp. 604-610.

Lee, J., Attila, C., Cirillo, S. L. G., Cirillo, J. D., and Wood, T. K. 2009. Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. *Microb Biotechnol.* v.2, pp. 75-90.

Lee, J-H., Wood, T. K., and Lee, J. 2015. Roles of indole as an interspecies and interkingdom signaling molecule. Trends Microbiol. v. 23, pp. 707-718. https://10.1016/ j.tim.2015.08.001707.

Liu, W., Røder, H. L., Madsen, J. S., Bjarnsholt, T., Søresen, S. J., and Burmølle, M. 2016. Interspecific bacterial interactions are reflected in multispecies biofilm spatial organization. *Front Microbiol.* v. 7, p. 1366. https://10.3389/fmicb.2016.01366.

Lopes, S. P., Machado, I. M., and Pereira, M. O. 2011. Role of planktonic and sessile extracellular metabolic by production *Pseudomonas aeruginosa* and *Escherichia coli* intra and interspecies relationships. *J Ind Microbiol Biotechnol.* v. 38, pp. 133-140.

Ma, Q., Qu, Y., Zhang, X., Liu, Z., Li, H., Zhang, Z., Wang, J., Shen, W., and Zhou, J. 2015. Systematic investigation and microbial community profile of indole degradation processes in two aerobic activated sludge systems. Scientific Reports. v. 5, p. 17674. https://doi 10.1038/srep17674.

Mahto, B., and Goel, S. 2008. Bacterial survival and regrowth in drinking water systems. *J Environ Sci Eng.* v. 50, pp. 3-40.

Mangwani, N., Kumari, S., and Das, S. 2015.Involvement of quorum sensing genes in biofilm development and degradation of polycyclic aromatic hydrocarbons by a marine bacterium *Pseudomonas aeruginosa* N6P6. *Clin Microbiol Infect.* v. 99, pp. 10283-10297.

Martins, V. V., Macareno, A. C., Gradella, D. G., and Stehling, E. G. 2014. Antagonism between clinical and environmental isolates of *Pseudomonas aeruginosa* against coliforms. *Water Suppy.* v. 14, pp. 99-106.

Mittal, S., Sharma, M., and Chaudhary, U. 2015. Biofilm and multidrug resistance in uropathogenic*Escherichia coli*. Pathog Glob Health. v. 109, pp. 26-29.

Molina-Santiago, C., Udaondo, Z., Cordero, B. F., and Ramos, J. L. 2017. Interspecies cross-talk between co-cultured *Pseudomonas putida* and *Escherichia coli.* Env Microbiol Rep. v. 9, pp. 441–448.

Nadell, C. D., Drescher, K., and Foster, K. R. 2016. Spatial structure, cooperation and competition in biofilms. *Nat Rev Microbiol.* v. 14, pp. 589-600.

O'Malley, Y. Q., Abdalla, M. Y., McCormick, M. L., Reszka, K. J., Denning, G. M., and Britigan, B. E. 2003. Subcelluar localization of *Pseudomonas aeruginosa* pyocianincytotoxity in human lung epithelial cells. *Am J Physiol Lung Cell Molec Physiol*. v. 284, pp. L-420-L430.

Oliveira, B. T. M., Barbosa, P. S. Z., Cavalcanti, T. G., Amaral, I. P. G., and Vasconcelos, U. 2019. Craft beer waste as substrate for pyocyanin synthesis. *J Pharm Biolol Sci.* v. 14, pp. 21-25.

Pagano, P. J., Buchanan, L. V., Dailey, C. F., Haas, J. V., Enk, R. A. V., and Gibson, J. K. 2004. Effects of linezolid on *Staphylococcal* adherence versus time of treatment. *Int J Antimicrob Agents.* v. 23, pp. 226–234.

Palmer, G. C., Jorth, P. A., and Whiteley, M. 2013. The role of two *Pseudomonas aeruginosa* anthranilate synthases in tryptophan and quorum signal production. *Microbiology.* v. 59, pp. 959-969.

Pffaler, M. A., Messer, S. A., and Coffmann, S. 1995. Comparison of visual and spectrophotometric methods of MIC endpoint determinations by using broth microdilution methods to test five antifungal agents, including the new triazole D0870. *J Clin Microbiol*. v. 33, pp. 1094-1097.

Price-Whealan, A., Dietrich, L. E., and Newman, D. K. 2007. Pyocyanin alters redox homeostasis and carbon flux through central metabolic pathways in *Pseudomonas aeruginosa* PA14. *J Bacteriol.* v. 189, pp. 6372-6381.

Rodrigues, L. B., Santos, L. R., Tagliari, V. Z., Rizzo, N. N., Trenhago, G., Oliveira, A. P., Goetz, F., and Nascimento, V. P. 2010. Quantification of biofilm production on polystyrene by *Listeria*, *Escherichia coli* and *Staphylococcus aureus* isolated from a poultry slaughterhouse. *Braz J Microbiol*. v. 41, pp. 1082-1085.

Romero, D., Taxler, M. F., López, D., and Kolter, R. 2011. Antibiotics as signal molecules. Chem Rev. v. 111, pp. 5492-5505. https://10.1021/cr2000509.

Skariyachan, S., Sridhar, V. S., Packirisamy, S., Kumargowda, S. T., and Challapilli, S. B. 2018. Recent perspectives on the molecular basis of biofilm formation by *Pseudomonas aeruginosa* and approaches for treatment and biofilm dispersal. *Folia Microbiol*. v. 63, pp. 413-432.

Smid, E. J., and Lacroix, C. 2013. Microbe-microbe interactions in mixed culture food fermentations. *CurrOpinBiotechnol.* v. 24, pp. 148-154.

Sun, X., Cai, P., Sørensen, S. J., Mortimer, M., Gao, C., Huang, Q., Wang, Y., Lin, X., Wu, Y., Zhu, D., and Chen, R. 2019. Interspecific interactions in dual-species biofilms of soil bacteria: effects of fertilization practices. *J Soil Sed.* https://10.1007/s11368-019-02500-6.

Tyc, O., Song, C., Dickschat, J. S., Vos, M., and Garbeva, P. 2017. The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. Trend Microbiol. v. 25, pp. 280-292. https://10.1016/j.tim.2016.12.002.

Vasconcelos, U., Das, P., Dias, D. S. B., Bonifacio, T. T. C. , Arruda, R. R. A., Oliveira, B. T. M., and Cavalcanti, T. G. In: Mitra, A. (ed.) Microbial biofilms current research and practical implications (2020) Caister Academic Press, Wymondham, UK, pp. 75-98, https://doi.org/10.21775/ 9781912530328.02.

Vasconcelos, U., Lima, M. A. G. A., and Calazans, G. M. T. 2010. *Pseudomonas aeruginosa* associated with negative interactions on coliform bacteria growth. *Can J Pure Appl* Sci.v. 4, pp. 1133- 1139.

Viana, A. A. G., Martins, R. X., Ferreira, G. F., Zenaide-Neto, H., Amaral, I. P. G., and Vasconcelos, U. 2017. *Pseudomonas aeruginosa* and pyocyanin negatively act on the establishment of Enterobacteriaceaebiofilm on a ceramic surface. Int J Eng Res Appl. v. 7, pp. 23-30.

Wang, D., Ding, X., and Rather, P. N. 2001. Indole can act as an extracellular signal in *Escherichia coli.* J Bacteriol. v. 183, pp. 4210-4216.

Zhang, W., Wang, H., Zhang, J., Chen, J., Shen, H., and Xie, P. 2019. Habitat species pools shape algal and bacterial communities and their correlations. Environ Microbiol Rep. v. 11, pp. 275-286. https://doi.org/10.1111/1758-2229.12754.

****